HATFIELD, BRIDGET E., Ph.D. Cortisol and Alpha-amylase Levels of Preschool Children While Attending Child Care: Relationships with Indicators of Classroom Quality. (2010)
Directed by Dr. Linda L. Hestenes. 102 pp.

Accumulating evidence suggests children enrolled in full-time child care often display afternoon elevations of the hormone cortisol, which is an indicator of stress. Another physiological measure of stress is alpha-amylase. Recent advances in immunoassays have allowed for the measurement of activity in the hypothalamic-pituitary-adrenal axis and the autonomic sympathetic nervous system from saliva, and measurement of both systems provides a more complete understanding of activity in the stress response system. This study is the first to examine both cortisol and alpha-amylase in children attending child care. The current study examined whether specific indicators of classroom quality were linked to cortisol and alpha-amylase output in preschool children. A diverse sample of Sixty-one preschool children nested in 14 classrooms of varying quality participated in this study. Classroom quality was assessed using numerous approaches to quality and child salivary cortisol and alpha-amylase were examined at eight times over two days. An afternoon elevation in cortisol was not evident in the current study; alpha-amylase and cortisol displayed a symmetrical relationship, supporting an additive model of functioning in the stress response system. Area under the curve with respect to ground was computed to reflect children's total output of cortisol and alpha-amylase while attending child care. Results indicated that children in classrooms with higher emotional support, increased language and interactions, and more available activities and materials demonstrated lower alpha-amylase output. Implications for professional development for early childhood teachers are discussed.

CORTISOL AND ALPHA-AMYLASE LEVELS OF PRESCHOOL CHILDREN WHILE ATTENDING CHILD CARE: RELATIONSHIPS WITH INDICATORS OF CLASSROOM QUALITY

by

Bridget E. Hatfield

A Dissertation Submitted to
The Faculty of The Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Greensboro
2010

Approved by

Linda L. Hestenes, Ph.D.
Committee Chair

To Mom, Dad, Rae, & Nat

You have been there for me every step of the way.

Much love and thanks.

APPROVAL PAGE

This dissertation has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

Committee Chair Linda L. Hestenes, Ph.D.

Committee Member Deborah Cassidy, Ph.D.

Marion O'Brien, Ph.D.

Danielle Crosby, Ph.D.

Karen La Paro, Ph.D.

6/2/10
Date of Acceptance by Committee

6/2/10
Date of Final Oral Examination

ACKNOWLEDGMENTS

A sincere thanks to the members of my dissertation committee; your passion, persistence, and grace have shaped me into the scholar I am today. To my advisor, Linda Hestenes, thank you for always supporting me. I could not have hoped for a better mentor, colleague, and friend.

Finally, to the child care centers, children, teachers, and families who participated in Project CCARE. Thank you.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

Humans experience stress daily, often triggered by situations such as having an argument with a friend or arriving late to work. The body responds to these stressors by activating the stress response system which enables survival and promotes adaptation to the environment by activating numerous biological systems (Loman & Gunnar, 2010). Simply, the stress response system helps humans manage arousal and stressors. This biological response to stress is adaptive and promotes survival; however prolonged exposure to repeated stressors or overactive or underactive stress response systems are maladaptive (Kyrou & Tsigos, 2009). The stress response system is immature throughout infancy and early childhood and is largely influenced by experiences, relationships, and interactions during this period (Levine, 1994; Meaney et al., 1996). Given the malleability of the stress response system as well as the association between the functioning of the stress response system and child outcomes, the current study focuses on individual and classroom influences on the developing stress response system in preschool children.

Psychobiology of Stress

The stress response system is constructed of two major components, the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic sympathetic nervous system (SNS). The biological response to a stressor originates in the central nervous system (CNS) and travels to the two principal components of the CNS: the SNS and the HPA axis (Gunnar & Quevedo, 2007). These systems converge at the hypothalamus where a behavioral response within the individual is signaled (Palkovits, 1987). The SNS creates epinephrine and norepinephrine providing adrenaline to focus attention, support vigilance and arousal (Gunnar & Quevedo), and activate the HPA axis. The HPA axis produces cortisol, a steroid hormone which impacts brain functioning and behavior (Bohus, de Kloet, & Veldhuis, 1982) as well as regulates important bodily functions and expressions such as the immune system *(*Palacios & Sugawara, 1982) and emotional expression (Oberlander et al., 2008). The activity in the HPA axis and SNS can be measured non-invasively via saliva (Granger et al., 2006; Granger, Schwartz, Booth, Curran, & Zakaria, 1999).

Salivary cortisol is a well-established measurement of activity in the HPA axis (Granger et al., 1999) which is directly present in saliva and is strongly correlated with serum cortisol levels (Charmandi, Tsigos, & Chrousos, 2005). Cortisol levels in individuals vary diurnally with levels highest in the morning and gradually declining throughout the day (Gunnar & Donzella, 2002). The measurement of the SNS system via saliva is not as straightforward as the HPA axis considering that epinephrine and norepinephrine are not directly present in saliva. Recent advances in immunoassays have identified salivary alpha-amylase as predictive of norepinephrine levels in humans (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996), and it is considered to be a surrogate biomarker for the SNS (Granger et al., 2006). Alpha-amylase also follows a diurnal pattern, decreasing 60 minutes after wake, increasing over the day peaking around

4:30pm, and then declining (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007). The above patterns of activity in the HPA axis and the SNS promote homeostasis within the stress response system (McEwen & Seeman, 1999). Because activity in the SNS and HPA axis are dependent on one another, the measurement of both systems provides a more complete understanding of the stress response system (Bauer, Quas, & Boyce, 2002; Granger & Fortunato, 2008).

The functioning of the stress response system is dictated by multiple factors including genetics, development, and the environment which shape an individual's ability to adapt and respond to stress (Charmandi et al, 2005; Clinton, Miller, Watson, & Akil, 2008; McEwen & Seeman, 1999). The stress response system is designed to endure a reasonable amount of activity due to stressors; however when the threshold is reached, deviations from the typical pattern of activity occur. For example, early adversity or chronic stress may alter the expected activity pattern of the stress response system (Saridjan et al., 2010) resulting in allostatic load. Allostatic load is manifested through hyperarousal (inability to "turn off" the systems), hypoarousal (inability to "turn on" the systems), or, when an individual is subjected to consistent stressors causing the systems to be in a constant state of reactivity and recovery (McEwen & Seeman). Over time, allostatic load in the HPA axis leads to deleterious effects on the body with links to memory deficits (Abercrombie, Kalin, Thurow, Rosenkranz, & Davidson, 2003), poor health outcomes (Volkmann & Weekes, 2006), and maladaptive emotional responses (Brake, Zhang, Diorio, Meaney, & Gratton, 2004). Specifically, elevated activity in the HPA axis is linked externalizing behaviors (Fortunato, Dribin, Granger, & Buss, 2008), anxiety, depression, and immune deficiencies (Greenspan, 2003). Further, preliminary evidence suggests increased activity in the SNS is related to externalizing behaviors and poor health (El-Sheikh, Mize, & Granger, 2005).

The Stress Response System in Early Childhood

Early childhood marks an important time frame in which the two systems are malleable and highly suggestible to environmental influences (Gunnar & Quevedo, 2007; Romeo & McEwen, 2006). The experiences in early childhood are likely to have a permanent impact on the stress response system as children who experience early life adversity display altered patterns of activity and/or elevated levels of cortisol (Charmandi et al., 2005; Gustafsson, Janlert, Theorell, & Hammarström, 2010; Nicholson, 2004). Specifically, caregiver-child interactions influence the development of stress response system as children with caregivers who are less nurturing and neglectful display deviant patterns of activity (Gunnar & Donzella, 2002). Beyond the interactions, the functioning of the stress response system is also influenced by genetics and recent theoretical perspectives argue that some children may be more susceptible to environmental influences than other children (Belsky, Bakermans-Kranenburg, & van Ijzendoorn, 2007; Boyce & Ellis, 2005). It is clear, however, that the experiences in early childhood have a lasting effect on the stress response system, impacting socioemotional development (Loman & Gunnar, 2010) and health (Charmandi et al., 2005). Thus it is important to identify the environmental influences and individual differences in early childhood that are associated with allostatic load, specifically elevated patterns of activity (hyperarousal) in the HPA and SNS.

Given that the majority of young children in the United States experience some form of child care, the characteristics and influence of this environment on the stress response system are of interest. In general, child care quality has been linked to development across a number of domains (Burchinal et al., 2000; NICHD ECCRN, 2001; Peisner-Fienberg et al., 2001). However, the influence of teachers and classroom quality on the stress response system as measured by both HPA axis and SNS function has yet to be explored. Thus, this study will explore influences on the stress response system in preschool children, identifying individual child and classroom characteristics that are associated with activity in the SNS and HPA axis.

CHAPTER II

THEORY

The following section describes Bronfenbrenner's bioecological model (Bronfenbrenner & Morris, 2006) in order to provide a foundation for exploring potential influences on the developing stress response system in young children. Under this framework, the classroom environment, interactions within the classroom, and individual differences are discussed. Building on the illustrations from the bioecological model, two theories, biological sensitivity to context (Boyce & Ellis, 2005, 2008) and differential susceptibility (Belsky, 1997, 2005; Belsky et al., 2007; Belsky & Pluess, 2009), are discussed to further explore how children may experience the environment differently, for better or for worse, with individual differences serving as a springboard for development.

Bioecological Model

Urie Bronfenbrenner developed the bioecological model (Bronfenbrenner & Morris, 2006) over most of his academic tenure. Between his publication in 1979, describing the ecological model, and the 1998 official presentation of the bioecological model, there were many reinventions, additions, and modifications which eventually led to the creation of the bioecological model. Due to the complexity of the theory, it is important when discussing his theory to identify the specific components of the biological model which frame the theoretical perspective (Tudge, Mokrova, Hatfield, & Karnik, 2009). The current study uses the bioecological model to understand the potential impact of quality in child care classrooms and child characteristics on the activity in the stress response system in preschool children. Within the bioecological model, development is influenced by characteristics of the person, other individuals, and the environment. Specifically, “In the bioecological model, development is defined as the phenomenon of continuity and change in the biopsychological characteristics of human beings, both as individuals and as groups” (Bronfenbrenner & Morris, p. 793). Using this framework influences on an individual’s activity in the stress response system will be understood via the Process-Person-Context-Time (PPCT) model—which serves as the foundation for the bioecological model. Within the PPCT time is continuous, with importance given to the early years (Bronfenbrenner & Morris). However, in the current study, time will not be specifically examined as the biological markers of the stress response system are only measured over two days, not allowing for change over time to be established. Thus, in the sections below, three proponents of the PPCT model, process, person, and context, are explored and provide a foundation to understand the influences on the stress response system in young children.

Process.

The process component, defined by Bronfenbrenner in proposition one as proximal processes, is considered to be the driving force of development. A proximal process is a contingent interaction between the developing individual and other persons or objects in the environment. These interactions, in order to promote growth and development, should be more complex over time, build on the individual's current ability, occur on a regular basis, and create enduring, identifiable patterns of interactions (Bronfenbrenner & Morris, 2006). Further, the form, direction, content, and power of the proximal processes should be considered along with a particular developmental outcome. In other words, the type, individual involvement, nature of, and the magnitude of the interaction should be considered when linking proximal processes to child outcomes. For example, if a child spends one month in a harsh, punitive, and nonstimulating child care classroom and then enrolls in a sensitive, enriching child care classroom for the next three years, the proximal processes in the low quality classroom will have a negligible effect on the child's development. Thus, the interactions that are consistent and prolonged in the child's life will have the largest impact on development. The interactions the child experiences in child care have the potential to markedly impact the development of the emerging physiological stress system (Gunnar & Quevedo, 2007).

In the current study proximal processes are examined in order to understand how specific aspects of the classroom may impact the stress response system in preschoolers. Proximal processes in the classroom take a variety of different forms such as peer-peer, child-environment, and teacher-child. Within this framework processes which are autonomy promoting, sensitive, language rich, and guided by the child's current ability may afford experiences in which the child feels comfortable to explore, respond, and learn. Experiences and interactions in the classroom have the potential to promote adaptive and maladaptive stress responses as they occur in an environment which is important and meaningful for the child (Granger & Fortunato, 2008). For example, research shows that children in classrooms where teachers provide small amounts of stimulation (i.e. verbal interaction) demonstrate aberrant patterns of cortisol throughout the day (Dettling, Parker, Lane, Sebanc, & Gunnar, 2000). Beyond the form, direction, power, and content of proximal processes, the interactions are also influenced by individual characteristics such as socioeconomic status, temperament, and ethnicity as well as characteristics of the context (e.g. structural quality of the environment, teacher education and training). Thus, characteristics of the person and context, in addition to proximal processes, are also explored within the bioecological model to fully understand development.

Person.

Bronfenbrenner (Bronfenbrenner & Morris, 2006) recognized the importance of individual characteristics as an influence on development, and it serves as the second "P" in PPCT. Person characteristics, specifically force, demand, and resource characteristics, impact the direction and form of proximal processes and contribute to individual differences. Demand characteristics are qualities of a person that promote or discourage immediate reactions from other individuals (Bronfenbrenner & Morris) such as gender, ethnicity, or age. These characteristics have direct implications concerning the availability and quantity of proximal processes as they are visually apparent to others. Resource characteristics focus on an individual's mental and emotional abilities as well as their social and financial resources (Bronfenbrenner & Morris), and are thus more difficult to induce from appearance or initial expectations. Lastly, force characteristics are active behavioral qualities that influence the duration and direction of proximal processes, and include individual characteristics such as temperament, persistence, and motivation.

Some evidence exists to argue that demand characteristics, specifically ethnicity and age, impact the development of the stress response system. Often African American children exhibit higher levels of alpha-amylase (Gordis et al., 2008; Hill-Soderlund et al., 2008) and lower levels of cortisol (Blair et al., 2008) compared to European American or Latino children. Thus, there is evidence that ethnicity may have an effect on activity in the SNS and HPA axis. Child age is important as alpha-amylase is not present in saliva until approximately 2 months of age and does not reach a diurnal pattern until 2 years (Davis & Granger, 2009). However, there is not currently sufficient evidence to indicate that resource characteristics, such as income or intelligence, contribute to the activity in the stress response system. Force characteristics play a vital role in the development of the stress response system. Two children may have similar demand and resource characteristics, but their developmental trajectories may vary considerably due to force characteristics as these characteristics influence duration and direction of proximal processes. Thus, force characteristics, such as reactivity, may play a salient role in understanding the development of the stress response system.

Aspects of temperamental reactivity such as approach behavior, negative affect, and positive affect have demonstrated a link to HPA or SNS activity (Dettling et al., 2000; Fortunato et al., 2008; Watamura, Sebanc, & Gunnar, 2002). Temperamental qualities also impact the proximal processes that occur in the classroom in that children with more difficult temperaments enrolled in low quality classrooms exhibit more behavior problems, impacting the availability of proximal processes (Crockenberg & Leerkes, 2005). Thus quality of proximal processes may be influenced by person characteristics. However, context also impacts proximal processes and is the final component of the PPCT model which will be investigated in this study.

Context.

Beyond proximal processes, the PPCT model postulates that context (i.e. characteristics of the environment) plays a role in an individual's developmental capacity. In the bioecological model, context is defined by four different levels of influence: microsystem, mesosystem, exosystem, and macrosystem. The microsystem is defined as a pattern of sustained experiences or relationships in which the child experiences face-to-face interactions that engage the child in proximal processes within a structured environment (Bronfenbrenner & Morris, 2006). The child care classroom serves as a microsystem for 6 million children under 5 (Johnson, 2002). The interrelationships among the child's home, child care, and other important settings (i.e. place of worship, neighborhood playground) are captured under the mesosystem. Specifically, the mesosystem comprises the interrelations among two or more microsystems (Bronfenbrenner, 1979). The exosystem is defined as "… one or more settings that do not involve the developing person as an active participant, but in which events occur that affect, or are affected by, what happens in the setting containing the developing person" (Bronfenbrenner, p. 25). Lastly, the macrosystem encompasses cultural, governmental, and state level restrictions and regulations that directly effect the child (Bronfenbrenner & Morris). Thus, factors in the environment removed from the child's control have direct implications on development. While all of these contexts are important to understand the development of the stress response system in children, only the impact of the microsystem is directly explored in the current study.

Bronfenbrenner (Bronfenbrenner & Evans, 2000) highlights additional contextual variables, chaos and crowding, as important aspects of context. Chaotic systems are environments, such as the home or classroom, that are characterized by unpredictable, irregular, and frenzied activity with high levels of sensory stimulation (Evans, 2006). Crowding is defined as the number of people per room or the available space for each individual (Evans). Environments that are chaotic and/or crowded may interfere with proximal processes as caregivers who reside in chaotic environments are less able to engage children with positive interactions, thus restricting developmental outcomes (Wachs, 1993). Additionally, chaotic and crowded environments are linked to elevated activity in the HPA axis and SNS (Evans & Saegert, 2000; Legendre, 2003).

Together the environment and proximal processes create the likelihood of two types of outcomes: competence and dysfunction (Bronfenbrenner, 2001). Competence refers to an individual's knowledge and skills which allow for successful management and facilitation in a variety of situations. Dysfunction, on the other hand, describes an individual who is unable to manage control over behavior across situations. In chaotic environments, children are more likely to display dysfunction which is often frequent and severe. The caregivers in these environments are often ill-equipped to appropriately manage the dysfunction. However, the bioecological model argues that in environments with more resources, behavioral manifestations of dysfunction are likely to be overlooked in favor of displays of competence. In these environments caregivers are more likely to encourage and respond to competence. With this view, disadvantaged children may seem to be at risk for developing poor behavior regulation as their environments promote displays of dysfunction. However, there is some evidence to suggest that if a disadvantaged child has the opportunity to experience an enriching child care environment, development may be positively impacted (Chryssanthopoulou, Turner-Cobb, Lucas, & Jessop, 2005).

The bioecological model helps to explain how the stress response system is influenced by proximal processes, context, and person characteristics. It provides an excellent foundation to understand the mechanisms by which the environment, temperamental characteristics, ethnicity, and characteristics of the context impact development of the stress response system. However, it has recently been hypothesized that the environment, proximal processes and personal characteristics may matter more for some children than others. The following section builds on the framework of PPCT as well as Bronfenbrenner's conceptions of competence, dysfunction and chaos, illustrating that these components may impact children differently.

Individual Differences and Susceptibility to Environments

The bioecological model examines development according to the PPCT model, arguing that process, person, context, and time combine to affect children. However, recent theoretical perspectives speculate that the environment may matter more for some children and less for others, a concept referred to as conditional adaptation. There is a growing body of evidence that some children, due to individual differences, may be more vulnerable and susceptible to both positive and negative environmental influences (Ouellet-Morin et al, 2009; Pluess & Belsky, 2009). The presence of such differences and interactions is illustrated through Boyce and Ellis' biological sensitivity to context (Boyce & Ellis, 2005) and Belsky's (1997, 2009) theory of differential susceptibility. Both of these theories postulate that environment matters more for particular children. However, the theories differ in how individual differences are determined by the environment.

Biological sensitivity to context.

Biological sensitivity to context (BSC; Boyce & Ellis, 2005) focuses on genetic and evolutionary predispositions which influence the impact the environment has on the stress response system. Through natural selection and evolution, humans have garnered skills to understand the nature of the environment they experience in infancy and are able to identify levels of stability, support, stress, and adversity. With this information, which is realized early in life, the stress response system adjusts accordingly following three major paths (Ellis, Essex, & Boyce, 2005) allowing children in each path to thrive in the environment in which he/she is born. Children who are exposed to acute, severe stressors, such as very stressful home or child care environments, poverty, or sustained parent-child conflict, develop highly reactive stress response systems so that the individual is able to manage the dangers and threats in the environment, promoting survival. Conversely, children exposed to marked nurturing and supportive environments also display highly reactive systems, allowing them to benefit from these environments by increasing their susceptibility to social support (Ellis et al.). Finally, children who experience neither extreme develop lower reactive profiles, as their environment is not extremely stressful or irrevocably secure. With respect to the stress response system, this creates a u-shaped relationship between environment and reactivity, with children experiencing environmental extremes developing highly reactive tendencies.

These reactivity profiles are best illustrated by the dandelion child (low reactivity) and the orchid child (high reactivity). The dandelion child is able to thrive in any context, seemingly resilient to any hint of adversity and is ambivalent to the environment. The orchid child, on the other hand, is highly sensitive to context and early experience. If the orchid child develops in an environment which is supportive and nurturing, the child will thrive. However, the child will likely suffer negative outcomes if subjected to prolonged neglect or high levels of stress. These biological reactive profiles reflect the notion that the environment assists in the development of reactivity. Thus, BSC views reactivity as a moderator of environmental influence on child outcomes, where reactivity is defined from the early environment.

However, Belsky's theory of differential susceptibility identifies highly reactive children at the behavioral and genetic level, arguing that some children may be more plastic and capable of adapting under optimal environmental conditions. Additionally, in direct opposition to BSC, differential susceptibility allows for reactivity to be determined by nature and nurture early in life arguing that both biological tendencies and environmental effects are capable of impacting development (Belsky, 2005). This premise highlights the major distinction between BSC and differential susceptibility and demonstrates the relevance of differential susceptibility when examining environmental influences on children's stress response system.

Differential susceptibility.

Differential susceptibility is based on the premise that children are not equally affected by the environment. The foundation for differential susceptibility lies mainly in evolutionary theory, which states that the primary purpose for all living things is reproduction. Natural selection aids in designing optimal reproductive fitness; thus, traits in humans are modified by natural selection, dispersing the most adaptable genes into future generations. Given the uncertain nature of the future, it is likely that parents unconsciously provide their offspring with a diverse array of traits, creating children that are variably suited for reproduction and allowing for diversification in genes across siblings. In simpler terms, children differ in their susceptibility to the rearing environment (Belsky, 2005).

Belsky first developed this theory to highlight the disparity of research investigating the positive effects of the environment for children. For instance, instead of quantifying individuals by varying degrees of vulnerability to negative environmental influences, the focus should be identifying children who are susceptible to positive and negative effects. Specifically, individual differences in reactivity serve as a moderator between the environment and behavior. For instance, environment may be disproportionally beneficial (or harmful) to highly reactive children (Crockenberg & Leerkes, 2005; Pluess & Belsky, 2009) but this relationship is only evident when an interaction of environment and reactivity predicts child behavior. Thus, it is imperative that these relationships are investigated to identify aspects of the environment that support positive outcomes for children.

At the individual level, children vary in their susceptibility stemming from biological and/or behavioral factors. Biologically, three genes, *DRD4* (a dopamine receptor), *MAOA* (monoamine oxidase-A) and, *5-HTT* (a serotonin transporter gene) are linked to potential gene by environment interactions (for a review see Belsky et al., 2009). Variants of these genetic markers appear to influence individual's susceptibility to the environment. For example, individuals with a short *5-HTT* on the surface, do not appear to be more likely to develop depression than those with the long *5-HTT*. However, when children with the short allele experienced severe maltreatment in the first 10 years of life, they were more likely to be diagnosed with major depressive disorder (Caspi et al., 2003). Similar studies have demonstrated a relationship between *MAOA* (Kim-Cohen et al., 2006) or *DRD4* (Bakersman-Kranenburg & van Ijzendoorn, 2006) predicting behavioral outcomes only when the moderator of environment is included. Lastly, recent twin studies indicate that infant cortisol activity was related to genetic and unique environmental factors for twins in the high family adversity group but not the low risk group (Ouellet-Morin et al., 2008, 2009). Thus, in support of differential susceptibility, genetic factors and heritability impact developmental outcomes only when certain environmental characteristics are experienced.

Beyond genetic influences, individual plasticity to the environment is also manifested in behavior. Temperament, a behavioral indicator of reactivity, dictates how individuals perceive and experience the environment (Belsky & Pluess, 2009). Children low in emotional negativity are less affected by a positive or negative caregiving environment, whereas children high in emotional negativity are significantly impacted by the early environment. Specifically, young children who are highly reactive benefit from a supportive, sensitive child care environment, exhibiting less behavior problems when compared to their counterparts in low quality environments *as well as* children who are low in reactivity (Pluess & Belsky). Conversely, highly reactive children exposed to low quality child care environments displayed more behavior problems. Thus, in this case, temperament moderates the impact child care quality has on behavior problems.

Differential susceptibility allows for children to be affected by the environment differently depending on a host of individual differences. The theory also argues that children will vary in their degree of plasticity where plasticity is best viewed on a continuum. Moreover, an individual's susceptibility is not general; rather, it is domain and outcome specific. For example, a child's emotional development may be impacted by a harsh, chaotic child care environment but not affect cognitive development. Thus, it is important to identify the aspects of the child care environment that significantly impact children's activity in the stress response system. Once these salient aspects are identified, the relationships between individual susceptibility to the environment and child outcomes can be further explored.

Together, the bioecological model, differential susceptibility, and BSC provide an extensive framework for understanding how the environment impacts development and how the effects may differ depending on individual differences. Without this framework, researchers may be over or underestimating the effect of, for example, the teacher-child relationship on child emotional development. In the following chapter, research examining the relationships between child care quality, temperament, and activity in the HPA axis and SNS is reviewed, highlighting the complexities among environment, individual differences, and the stress response system.

CHAPTER III

LITERATURE REVIEW

In the past 20 years, center-based care has emerged as a relevant and integral environment for young children. This is particularly true for 4-5 year old children as 75% of this age group is enrolled in some type of non-maternal care in the United States (Cappizzano & Adams, 2000) with the majority of these children in center-based care (Johnson, 2002). The quality of child care is linked to academic, social, and emotional outcomes for children and adolescents. An abundance of evidence supports the relationship between high quality child care and children's academic preparedness for school (Burchinal et al., 2000), language development (NICHD ECCRN, 2003), and math skills (Burchinal et al., 2008). However, the relationship between child care quality and socioemotional outcomes is unclear and often yields contradictory findings (NICHD ECCRN, 2001, 2003). The inconsistent conclusions may be due to the lack of research devoted to understanding the underlying physiological and biological mechanisms by which the quality of child care effects child social and emotional functioning. Measurement of child social and emotional behaviors often rely on teacher or parent report and observations of child behaviors, largely depending on the views of others to interpret behavior. Salivary cortisol and alpha-amylase provide a mechanism to measure individual experience via activity in the HPA axis and SNS. In order to understand individual differences in the stress response system, both the activity in the HPA axis and SNS must be investigated as the two systems are complementary (Bauer et al., 2002). To date, however, research has focused solely on cortisol as a measure of the stress response system to understand child experience within the classroom environment.

The biolecological model (Bronfenbrenner & Morris, 2006), differential susceptibility (Belsky, 2005), and biological sensitivity to context (Boyce & Ellis, 2005) provide a foundation to explore influences on the developing stress response system as both focus on the importance of exploring individual differences while also examining contextual contributions. In the following section individual and contextual influences within the relationship between the stress response system and child care are examined. First, the contribution of the child care context to HPA axis activity is examined. Specifically, contextual differences between home and child care are illustrated, focusing on how discrepancies between home and classroom may lead to higher activity in the stress response system. Then, aspects of quality specific to the classroom, such as available materials and teacher-child interactions, are highlighted with respect to the stress response system. Finally, the relationships between individual reactivity and activity in the HPA axis and SNS are explored.

Contextual Influences on the Stress Response System

In the mid 1980's there was an influx of women returning to the workforce (Hill, Waldfogel, Brooks-Gunn, & Han, 2005) and with it an explosive need for out-of-home care. Of particular concern was the potential detrimental effect child care would have on the child, given the absence of the primary attachment figure, and the integration into a new environment (Belsky & Steinberg, 1978). Thus, children's experiences in child care were investigated due to the notion that group care would be challenging and stressful for young children, and this experience would markedly differ from experience at home (Dettling, Gunnar, & Donzella, 1999). Early studies examining the stress response systems simply focused on comparing children's levels of cortisol at home and during child care examining differences between the two contexts. Recent studies have moved beyond comparison to understanding the mechanisms and indicators of classroom quality that are influencing the stress response system.

Comparison of home and classroom environments.

Dettling and colleagues (1999) were among the first to examine differences in children's cortisol at home and in child care. They collected morning and afternoon cortisol levels for 70 preschool and school aged children from two high quality centers. Results indicated that afternoon cortisol levels were higher in child care when compared to home levels, and this effect was stronger for preschool children as 80% of 3 year olds and only 60% of 4 year olds experienced higher afternoon cortisol in child care. Further evidence of elevated afternoon cortisol in child care was established with a meta-analysis of seven studies with home and child care data (Vermeer & van IJzendoorn, 2006). Results from this meta-analysis concluded that the effect size (n = 303) is r = .18, implying that context (home versus child care) contributes 18% to individual rise in cortisol. Thus, children are likely to display a rise in cortisol throughout the day while in child care and these children do not display this rise at home. Beyond the general contextual difference, research has focused on two specific differences between home and child: napping and social demands.

One of the original variables thought to influence the rise in cortisol at child care was the quality and frequency of napping. Cortisol production resembles a typical sleep-wake cycle by approximately the first year of life (Spangler, 1991); however napping temporarily disrupts the typical pattern as cortisol rises after nap and then returns to pre-nap levels within 45 minutes after wake (Larson, Gunnar, & Hertsgaard, 1991). When compared to napping at home, children at child care may not receive an adequate nap, or the quality of rest may not be similar. However, current research does not support napping as an important influence on children's afternoon cortisol levels. Napping factors such as the duration and perceived quality of sleep are not linked to cortisol levels in preschool children (Lisonbee et al., 2008; Ouellet-Morin et al., 2009; Watamura et al., 2002).

In contrast to the home environment, children in child care are expected to navigate through numerous social bids, challenges, and exchanges throughout the day (Dettling et al, 1999). The increased repeated social demands associated with child care are likely to influence activity in the stress response system given that these events may be stressful for the child. Research indicates that children manage these social demands in two ways. First, children who are more socially competent and able to navigate peer play are able to manage this arousal and display typical cortisol patterns (Granger, Stansbury, & Henker, 1994; Gunnar, Tout, de Hann, Pierce, & Stansbury, 1997; Watamura, Donzella, Alwin, & Gunnar, 2003). On the other hand, children who avoid interaction and primarily engage in onlooking or unoccupied play behaviors demonstrate lower cortisol levels (Tout, de Haan, Campbell, & Gunnar, 1998). The absence of social interactions and link to cortisol levels is also visible when comparing cortisol of infants and toddlers. Toddlers, who have more opportunities to engage in social interactions, are more likely to display an increase in cortisol levels at child care than infants (Watamura et al.). Thus, although napping at child care is not linked to cortisol levels, the increased availability of social interactions and the child's ability to navigate these bids for attention are emerging as influences on cortisol levels. The nature and facilitation of these interactions within the classroom may be a function of the classroom environment and teacher characteristics. Thus, the quality of the classroom must also be examined to understand influences on the developing stress response system.

Child care quality.

Quality in the child care classroom is broadly defined as aspects of the environment and experiences in the classroom that foster growth and development for children (Layzer & Goodman, 2006). One measure designed to assess global quality, the Early Childhood Environmental Rating Scale (ECERS-R; Harms, Clifford, & Cryer, 2003), is often utilized in the stress literature to capture quality of the classroom. It focuses on aspects of the classroom such as the materials, language exposure, developmentally appropriate discipline and guidance, and safety to define global quality. Many studies that examine the relationship between child care quality and cortisol levels recruit participants from centers rated by the ECERS-R as good or excellent (Rappolt-Schlichtmann et al., 2009; Tout, et al., 1998; Watamura et al., 2003). Overall, children from classrooms of good or excellent quality, as rated by the ECERS-R, experience a rise in cortisol throughout the day (Rappolt-Schlichtmann et al.; Watamura et al., 2002; Watamura et al., 2003), and this rise conflicts with the expected decline in cortisol throughout the day. Given the complex nature of the classroom, a global measurement of quality may not be the distinctive enough to specifically relate to children's socioemotional development. Instead, single aspects of classroom quality may impact children's development with varying effectiveness. For example, teacher's instructional skills are often predictive of child cognitive and academic skills while a close teacher-child relationship is a strong predictor of social competence, regulation of behavior (Peisner-Fienberg et al., 2001), and physiological reactivity (Lisonbee et al., 2008). Thus, it is likely that activity in the stress response system is related to different components of the quality of the classroom environment. The paragraphs below review the relationship between the two categories of child care quality, structural and process, and their relationship to activity in the HPA axis.

Structural quality is conceptualized as program characteristics (e.g. center's belief statement, staff characteristics), classroom characteristics (e.g. teacher-child ratio, physical materials in the classroom), and caregiver characteristics (e.g. teacher training and experience) (Layzer & Goodman, 2006). Structural quality refers to elements of the classroom that are constant, materialistic in nature, exist without interactions, and form the foundation for potential learning experiences for children. Three common indicators of structural quality: group size, square feet/individual, and teacher preparation have demonstrated a relationship with higher cortisol levels in young children.

The HPA axis generally interprets crowding or large groups as stressors and responds with elevated cortisol levels (Lisonbee et al., 2008; Martimportugués-Goyenechea & Gómez-Jacinto, 2005; Rappolt-Schlichtmann et al., 2009). In the child care setting, specific structural components of the classroom such as, group size, square feet/individual, and teacher preparation are related to cortisol levels in preschool children. Specifically, cortisol levels are lower in classrooms with more than 16 ½ feet^2 of useable play space per child and those with less than 15 people (Legendre, 2003). Thus, classrooms with more space per child are able to support activity in the HPA axis evident through lower cortisol levels throughout the day. Regarding teacher preparation, cortisol levels are lower for children in classrooms with a parent orientation and teachers who are invested in protecting the health and safety of each child (Sims, et al., 2006). Thus, even though classrooms with high global quality often display elevated cortisol levels in the afternoon (Rappolt-Schlichtmann et al.; Tout, et al., 1998; Watamura et al., 2003; Watamura et al., 2002), specific structural features of the classroom are related to typical cortisol.

Process quality encompasses the interactions and experiences that occur in the classroom which require active human interaction and intent (Cassidy, Hestenes, Hansen, Hedge, Shim, & Hestenes, 2005; Cryer, 1999; Phillipsen, Burchinal, Howes, & Cryer, 1997). Under this definition of quality, the teacher serves as a model for appropriate behaviors, guides instructional interactions, and provides a child-centered classroom. A sensitive, responsive caregiver provides a buffer against elevated cortisol, and a child is able to express and process emotion without impacting the HPA axis (Blair et al., 2008;

Gunnar & Donzella, 2002). Research with rodents maintains that responsive, sensitive, and attentive caregivers play a central role in sustaining low cortisol levels in the first year of life (Gunnar & Donzella). Additionally, marital conflict and quality of the mother-child relationship is linked to elevations in children's cortisol levels (Pendry & Adam, 2007; Rappolt-Schlichtmann et al., 2009). Thus, the teacher-child interactions and emotional climate of the classroom are likely to impact cortisol levels while children are in child care.

The mechanisms by which specific components of process quality impact cortisol levels have not been explored in depth as relatively few studies have examined links between cortisol and classroom processual quality. However, preliminary evidence reveals that classroom indicators of process quality such as increased opportunity for stimulation and classroom cohesion are linked to lower cortisol levels in young children. The Observational Ratings of the Caregiving Environment (ORCE; NICHD ECCRN, 1996), an observational measure of process quality, was designed to assess the quantity and quality of the care received by the child from the caregiver in child care settings. It examines the frequency of verbal teacher-child interactions, quantity of interactions that promote play, learning, or guidance, and an overall rating of caregiver sensitivity. Often the three scales are standardized and combined to create a composite of stimulation for each child in the classroom. Children who experience higher levels of stimulation, as measured by the ORCE, display lower cortisol levels which mirror patterns of cortisol activity at home (Dettling et al., 2000). A modified version of the ORCE (M-ORCE; Gunnar, Kryzer, Phillips, & Vandell, 2001) captures two additional variables measuring the degree of cohesion in the classroom environment as well as child reactivity. In early childhood, children in classrooms with higher positive cohesion displayed cortisol patterns that decreased throughout the day (Watamura, Kryzer, & Robertson, 2009). Additionally, the Classroom Assessment Scoring System (CLASS) is a measure of process quality which was created from the ORCE. Given these preliminary findings and improved measurement techniques, it is important to further investigate the relationships between specific indicators of process quality and the activity in the stress response system.

These results indicate that sensitive, responsive, and warm classroom environments are related to the expected decline in cortisol throughout the day. However, both classroom quality and child negative affect contribute to an increase in cortisol (Dettling et al., 2000). Also, research suggests that individual reactivity is associated with increased cortisol throughout the day. Thus, children bring individual differences to the child care classroom and these variables are also imperative to understand the interplay between context, person, and the stress response system.

Individual Reactivity

Reactivity captures an individual's activity in the motor, affective, and sensory response system in response to a stressor (Rothbart, Ahadi, Hershey, & Fisher, 2001). There are individual differences and behavioral patterns which conceptualize reactivity, and the stress literature focuses two of these constructs: negative affect and emotion regulation. Individual differences in negative affect are linked to cortisol levels. With respect to activity in the SNS, there is preliminary support that emotion regulation is related to alpha-amylase levels. Thus, both are discussed in the section below.

Negative affect is characterized by high levels of discomfort, anger/frustration, and sadness coupled with low levels of soothability (Rothbart et al., 2001). There is accumulating evidence that negative affect, measured from parent report or behavioral observation, is linked to higher cortisol levels in young children even after accounting for quality of context (Dettling et al., 2000; Lisonbee et al., 2008; Watamura et al., 2002). In general, highly reactive children display higher cortisol levels after experiencing a stressor or an emotion eliciting event (Blair et al., 2008; Fortunato et al., 2008). Further, children who demonstrate high frequencies of negative affect are more likely to be diagnosed with social or behavioral problems (for a review see Nigg, 2006). Given the parallelism between behavioral problems and cortisol (El-Sheikh et al., 2008) examination of this relationship warrants further scrutiny.

Emotion regulation captures an individual’s ability to manage emotional arousal and stressors (Calkins & Fox, 2002). The relationship between young children’s ability to manage emotional arousal and cortisol levels is not clear. Some research finds that children with fewer emotion regulation strategies display higher cortisol levels in child care (Gunnar et al., 1997), while other evidence illustrates a positive relationship between emotion regulation and cortisol (Spinrad et al., 2009). This ambiguity may be linked to a third factor, activity in the autonomic sympathetic nervous system (SNS). Other measures indicative of SNS functioning, such as respiratory sinus arrhythmia (Porges, Doussard-Roosevelt, & Maiti, 1994), are associated with appropriate emotional reactivity and control (Calkins & Howse, 2004). Thus, it is likely that salivary alpha-amylase may demonstrate a relationship with child emotion regulation and preliminary evidence supports this theory (Spinrad et al.).

The evidence suggests that individual reactivity and the quality of the classroom environment are key factors necessary to understand children's cortisol levels in child care. Activity in the HPA axis is influenced by negative affect, while emotion regulation is tentatively linked to alpha-amylase levels. Further, child ethnicity is linked to differences in baseline cortisol and alpha-amylase levels in children with African American children displaying higher levels of alpha-amylase (Gordis et al., 2008; Hill-Soderlund et al., 2008) and lower levels of cortisol (Blair et al., 2008) compared to European American or Latino children. Globally, classroom quality exhibits a relationship to cortisol levels, although the relationships between specific indicators of quality and their relationship to cortisol and alpha-amylase levels are unknown. Contributing to the uncertain relationship between classroom quality and cortisol is the homogenous sample that characterizes most of the current work (Vermeer & van IJzendoorn, 2006). Geoffroy and colleagues (2006) argue that future research should incorporate a range in classroom quality instead of using quality as an analytic precursor. Lastly, recent research supports the dual nature of the central nervous system, arguing that symmetrical activity among the HPA axis and SNS is optimal (Bauer et al., 2002; El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008; Gordis, Granger, Susman, Trickett, 2008). Previous research has not explored the contribution of salivary alpha-amylase to children's cortisol levels in child care.

Aims and Hypotheses

The primary aim of this dissertation is to parse out the indicators of classroom quality, identifying the most salient influences on cortisol and alpha-amylase output for preschool children. These aims are investigated using classrooms with diverse levels of classroom quality and family demographics. The inclusion of alpha-amylase and cortisol provides a foundation to better understand how specific child and classroom characteristics link to activity in the stress response system. Specifically, the relationships between classroom quality and child reactivity are explored separately for cortisol and alpha-amylase in order to better understand the unique contribution of each biological marker. Further, this research contributes to the understanding of how the HPA axis and SNS work together to regulate stress in children. The following research questions and hypotheses are explored in this study:

Q1). What do children's patterns of cortisol and alpha-amylase look like across the day at child care?

H1.1. Children will show a decline in cortisol throughout the day.

H1.2. Children will show an increase in alpha-amylase throughout the day.

Q2.) Does child ethnicity or negative affect predict children's overall output of cortisol?

H2.1. Children with higher negative affect will demonstrate higher cortisol output.

H2.2. Children identified as African American or mixed race will have lower cortisol output than Latino or Caucasian children.

Q3). Does classroom quality (level 2 variable) predict differences in children's total cortisol output? Further, after identifying any main effect of classroom quality, does the relationship between classroom quality and cortisol change as a function of negative affect?

H3.1. Children in classrooms with higher Emotional Support will demonstrate lower total cortisol output.

H3.2. Children in classrooms with higher Classroom Organization will demonstrate lower total cortisol output.

H3.3. Children in classrooms with higher Instructional Support will demonstrate lower total cortisol output.

H3.4. Children in classrooms with higher Activities and Materials will demonstrate lower total cortisol output.

H3.5. Children in classrooms with higher Language/Interactions will demonstrate lower cortisol output.

H3.6. Children in classrooms with more space available per child will demonstrate lower cortisol output.

H3.7. Children exhibiting higher negative affect will demonstrate lower total cortisol output only in classrooms with higher Emotional Support.

H3.8. Children exhibiting higher negative affect will demonstrate lower total cortisol output only in classrooms with higher Classroom Organization.

H3.9. Children exhibiting higher negative affect will demonstrate lower total cortisol output only in classrooms with higher Instructional Support.

H3.10. Children exhibiting higher negative affect will demonstrate lower total cortisol output only in classrooms with higher Activities and Materials.

H3.11. Children exhibiting higher negative affect will demonstrate lower total cortisol output only in classrooms with higher Language/Interactions

H3.12. Children exhibiting higher negative affect will demonstrate lower total cortisol output only in classrooms with more space available per child.

Q4.) Does child ethnicity or emotion regulation ability predict children's overall output of alpha-amylase?

H4.1. Children with lower emotion regulation skills will demonstrate higher alpha-amylase output.

H4.2. Children identified as African American or mixed race will have higher alpha-amylase output than Latino and Caucasian children.

Q5). Does classroom quality (level 2 variable) predict differences in children's total alpha-amylase output? Further, after identifying any main effect of classroom quality, does the relationship between classroom quality and alpha-amylase change as a function of child emotion regulation?

H5.1. Children in classrooms with higher Emotional Support will demonstrate lower total alpha-amylase output.

H5.2. Children in classrooms with higher Classroom Organization will demonstrate lower total alpha-amylase output.

H5.3. Children in classrooms with higher Instructional Support will demonstrate lower total alpha-amylase output.

H5.4. Children in classrooms with higher Activities and Materials will demonstrate lower total alpha-amylase output.

H5.5. Children in classrooms with higher Language/Interactions will demonstrate lower alpha-amylase output.

H5.6. Children in classrooms with more space available per child will demonstrate lower alpha-amylase output.

H5.7. Children exhibiting lower emotion regulation skills will demonstrate lower total alpha-amylase output only in classrooms with higher Emotional Support.

H5.8. Children exhibiting lower emotion regulation skills will demonstrate lower total alpha-amylase output only in classrooms with higher Classroom Organization.

H5.9. Children exhibiting lower emotion regulation skills will demonstrate lower total alpha-amylase output only in classrooms with higher Instructional Support.

H5.10. Children exhibiting lower emotion regulation skills will demonstrate lower total alpha-amylase output only in classrooms with higher Activities and Materials.

H5.11. Children exhibiting lower emotion regulation skills will demonstrate lower total alpha-amylase output only in classrooms with higher Language/Interactions

H5.12. Children exhibiting lower emotion regulation skills will demonstrate lower total alpha-amylase output only in classrooms with more space available per child.

CHAPTER IV

METHODS

Participants

Settings.

The classrooms that served as data collection sites for this study were selected from child care centers who participated in North Carolina's Division of Child Development (DCD) Star Rated License. Using this database, 100 child care centers were randomly selected from one metropolitan county. Under the Star Rated License, child care centers in North Carolina are awarded one to five stars based upon program standards and education; thus, it was possible to group centers into high quality or low quality categories. This classification process led to 50 centers in the high quality category (4 or 5 stars; M = 4.32) and 50 in the low quality category (1, 2, or 3 stars; M = 2.38). Of the original 100 child care centers, 39% did not have an age appropriate classroom, 15% of the centers were too busy to participate, 12% did not respond, 8% refused to participate, 3% were never contacted (maximum sample size was reached), 9% agreed to participate but failed to obtain enough parent consents, and 14% participated. The final sample included five lower quality centers (M = 2.8 Star rating) and nine higher quality centers (M = 4.33 Star rating); see Table 1 for demographic information by STAR rating. Sixteen teachers participated in the study which included 12 lead teachers and four co-teachers. The teachers were all female (*M* = 33 years, *SD* = 9.88) with 21% African American, 72% Caucasian, and 7% mixed race and some experience in the field of early care and education (*M* = 10.33 years, *SD* = 6.97). Eight teachers held a bachelor's degree, two teachers had an associate's degree, five teachers either earned a CDA, a North Carolina Early Childhood Credential, or attended some college, and one teacher was a high school graduate . Seven of the teachers with a bachelor's or a master's degree majored in child development or early childhood education. Seven classrooms in this study were More at Four programs; five were situated in child care centers and two in Head Start centers. The remaining seven classrooms were teacher-defined as a preschool or pre-kindergarten classroom in a child care facility.

Children.

Sixty-six typically developing preschool children were recruited for participation. However, four children were deleted from the sample as the parent failed to return the questionnaires (*n* = 1) or the questionnaires were completed incorrectly (*n* =2); one child refused to participate in saliva collection (explained in following section) resulting in a final sample of 62 children (*M* = 53.92 months; *SD* = 5.39; 51% female). Child race was identified by the parent (43% Caucasian, 35% African American, 3% Latino, and 18% mixed race). The children were from a range of economic backgrounds (24% earned less than $24,000/year, 36% earned between $24,000-$47,999/year, 16% earned between $48,000-$83,999/year, and 24% earned over $84,000/year) and the majority (62%) of children lived in married households. Fifteen percent of families reported using child care subsidy and the average child began attending some form of child care at 16 months of age (range 1 month to 58 months). Children were enrolled in the classroom where data collection took place for at least two months (*M* = 5.74, *SD* = 6.21) before the start of the study and spent an average of 39.61 hours (*SD* = 6.25) in child care each week.

Procedures

Center directors were initially contacted by mail and invited to participate. After the letter was received, up to three follow-up phone calls were placed by the author to the director to provide additional information, answer questions, and to determine if the center had an eligible classroom. Classrooms were eligible if the center contained a full-time classroom for at least 8 children who were between 36 and 59 months of age, and if the teacher for that classroom had served as the lead teacher for at least 8 weeks. Following approval from the director, an informational meeting was arranged with the director and eligible teacher(s). During this meeting teacher and director consent was obtained and parent recruitment packets were given to the teacher to distribute to all families in the classroom. Interested parents returned the consent form, and child eligibility was determined using the following criteria: enrolled in the classroom where data collection took place for at least 8 weeks, attended child care for at least 25 hours a week, typically developing, from English-speaking households, had not taken an oral steroid in the past 30 days (elevates salivary cortisol levels; Granger et al., 2006), and between 36 and 59 months of age during data collection. If more than five children in a classroom were eligible, then five were randomly selected to participate. In each of the 14 classrooms, three to five children (*M* = 4.43) participated. Parents (92% mothers, 5% fathers, 3% other) completed a variety of questionnaires describing child behavior and family demographics and answered a brief three item questionnaire assessing the child's typical day (i.e. quality of sleep, morning routine) as well as provided information about medications administered during classroom data collection. Parents were given a $20 gift card to Target after completion of these questionnaires. Teachers completed questionnaires regarding child behaviors in the classroom and basic demographics. For classrooms with co-teachers, demographics were collected on each teacher, and teachers completed the child behavior questionnaire together. The lead teachers or co-teachers each received a $30 gift card to Target for participating in this study.

Classroom assessments and child saliva collection occurred over two days while the child attended child care. Classroom assessments were conducted by the author and a graduate research assistant in the morning of the first day of data collection. Saliva was collected at four time points each day (shortly after arrival, twice more before lunch, and in the late afternoon). Before the very first collection, each participating child was approached by the author approximately 10 minutes before the initial collection to let the child know that they were invited to play a game with the author today. Then, the children were gathered in a quiet corner of the classroom and asked to sit in a small circle. The author explained to the children that they had been given permission by whoever takes care of them (e.g. mother, father, grandmother) to play some games with the author. The children were shown the Sorbettes® (the saliva collection device; it resembles a small triangle sponge on a stick) and told that it would tell the author how they feel in the classroom (e.g. happy, sad, angry). Then, the children were asked if they would like to participate, and assent from the child was obtained before collecting saliva.

The goal was to leave the Sorbettes in each child's mouth for 2 minutes and a minimum of 1 minute; however the child was allowed to stop at any time. All samples assayed were from a saliva collection period of at least 1 minute. After each saliva collection, the children were allowed to choose a sticker, and at the end of each day of data collection, the children took a small toy home.

Measures

Classroom quality.

Multiple assessment approaches were employed to define dimensions of classroom quality. The Early Childhood Environmental Rating Scale-Revised (ECERS-R; Harms et al., 2003), the Classroom Assessment Scoring System Pre-K (CLASS Pre-K; Pianta, La Paro, & Hamre, 2007), and classroom size measured as square foot per individual provided classroom level indicators of quality from structural and process perspectives.

The ECERS-R (Harms et al., 2003) measures global classroom quality and includes items such as materials for dramatic play, use of language, space for adults, and interactions among children. A recent factor analysis of the ECERS-R yielded 2 factors, Materials and Activities and Language/Interactions, which account for 69% of the variance in the items (Cassidy et al., 2005). The Materials and Activities factor (items 3, 5, 15, 19, 20, 22, 24, 25, and 26) evaluates the range of toys and types of activities available to the children and serves as one indicator of classroom structural quality. This factor has demonstrated acceptable reliability (α = .88); alphas above .70 indicate the measure displays adequate reliability (Cronbach, 1951). The Materials and Activities factor is highly correlated with the full ECERS-R score (*r* = .76; Cassidy et al.). The Language/Interactions factor (items 17, 18, 30, 31, 32, 33, and 36) defines the variety of teacher-child interactions and use of language in the classroom, and is used in this study as one indicator of classroom process quality. This factor has also exhibited acceptable reliability (α = .81) and is highly correlated with the full ECERS-R score (*r* = .81; Cassidy et al.). ECERS-R data was collected by one of two graduate students on the first day of data collection. One of the data collectors previously served as an assessor for the North Carolina Rated License Assessment Project and trained the second graduate student to 93.75% agreement (based on scoring within one point of the master coder). In the current study Activities and Materials and Language/Interactions factors demonstrated acceptable reliability (α = .84; α = .89), respectively.

The CLASS Pre-K (Pianta et al., 2007) measures classroom process quality by using observation of classroom behaviors. It focuses on ten dimensions which are collapsed into three domains (1) Classroom Organization, (2) Emotional Support, and (3) Instructional Support. Classroom Organization, comprised of the dimensions of behavior management, productivity, and instructional learning formats focuses on the predictability of classroom routines, management of behavior, and the teacher's facilitation of learning opportunities. Emotional Support, defined by the dimensions of positive climate, negative climate, teacher sensitivity, and regard for student perspective, rates the quality of the relationships, support for child's autonomy, and teacher's awareness and responsiveness to individual needs. Instructional Support, which includes the three dimensions of concept development, language modeling, and quality of feedback, assesses the use of conversation as a mechanism to promote language and teacher-child engagement in higher-order thinking skills and scaffolding. Classrooms are observed for 20 minutes and then all of the 10 dimensions are coded using a seven point Likert scale; typically at least 5 full cycles are completed. In previous research, the measure has demonstrated acceptable reliability (α = .85; La Paro, Pianta, & Stuhlman, 2004) and is only moderately correlated with the total ECERS-R (r = .52; La Paro et al.). All CLASS Pre-K observations were conducted by the author, who is a trainer for the CLASS and completes yearly reliability assessments on the CLASS. In the current study, the three domains demonstrated acceptable reliability (α = .87 to .89).

Space available per individual was calculated with the following equation: total classroom area/average number of persons, to provide an indicator of average square footage per individual, including teachers and children. Total number of individuals (teachers and children) was calculated during each cycle of the CLASS, and the total was averaged across cycles (M = 13.75, SD = 3.50). The total area of the classroom was measured on either day one or day two of data collection (M = 729.65ft^2, SD = 136.84). Higher scores indicate more square footage available per individual.

Child reactivity.

Parents rated child behavioral reactivity on a 7 point Likert scale (1 = extremely untrue, 7 = extremely true) scale using a reduced version (130-items) of the Child Behavior Questionnaire (CBQ; Rothbart et al., 2001). The CBQ is accepted as a reliable and valid measure of child temperament and reactivity, and the subscales consistently demonstrate acceptable reliability (.67 to .94; Ahadi, Rothbart, & Ye, 1993). Parent report of child negative affect is often correlated with teacher report of child negative affect (*r* = .23; Dettling et al., 1999). The superfactor Negative Affectivity (Ahadi et al.; Rothbart et al.) was computed from 5 subscales (discomfort, sadness, fear, anger/frustration, and reversed soothability) and demonstrated acceptable internal consistency (α = 74).

Teachers completed the Emotion Regulation Checklist (ERC; Shields & Cicchetti, 1997), which measures a child's ability to regulate emotions in the classroom on a 4 point Likert (1 = never, 4 = always). In the current study, the emotion regulation subscale was calculated from eight items (Shields & Cicchetti) such as (1) is a cheerful child, (2) is empathic towards others; shows concern or sadness when others are upset or distressed, and (3) can say when she/he is feeling sad, angry, mad, fearful, or afraid. The subscale has been used in other studies and is considered a reliable and valid measure of emotion regulation (Keane & Calkins, 2004; Shields & Cicchetti, 1998). It displayed acceptable reliability in the current study (α = .73).

Saliva collection and preparation.

Saliva samples were collected while the children attended child care, with the intent to collect 8 samples from each child over two days. Samples were collected three times in the morning and once in the afternoon on both days. The mean time for the first collection was 8:29AM (range 8:11 to 8:49AM), the second was 10:31AM (range 10:00 to 11:21AM), the third was 11:30AM (range 11:06-12:03), and the final collection was 3:27PM (range 1:53-4:41PM). The extended range of time for the fourth collection is due to two classrooms ending their day at 2:00PM. In the remaining 12 classrooms, the fourth collection occurred later in the afternoon (*M* = 4:13PM, range 3:05 to 4:41). An effort was made to collect saliva at approximately the same time on both days at each of the four collection points for children in the same classroom. For example, if the second sample on day 1 was collected at 10:02, an attempt was made to collect the second sample on day 2 at 10:02. Due to changes in classroom schedules, it was not always possible to collect at the exact same time. However, 95% of the samples were collected within 30 minutes of the intended time and the remaining 5% within 60 minutes. Saliva was collected by using two Sorbettes® (hydrocellulose microsponges), a reliable collection material for collecting both cortisol and alpha-amylase (Harmon, Hibel, Rumyantseva, & Granger, 2007; Granger et al., 2006). The samples were transported on ice and stored in a household refrigerator (Kirschbaum & Hellhammer, 1989; Rohlder & Nater, 2009) for no more than three weeks and then shipped to Salimetrics (State College, Pennsylvania) on dry ice where they were stored frozen at -80°C until assayed.

Determination of salivary analytes.

Cortisol and alpha-amylase.

Samples were assayed for cortisol using an enzyme immunoassay. The assays were performed in singleton on 25µl of saliva, and when possible classrooms were assayed in the same batch. The mean intraassay coefficient of variation (CV) for cortisol was 3.71% (1% to 10% is considered acceptable for cortisol and alpha-amylase; Granger et al., 2006). Saliva samples of 10µl were assayed for alpha-amylase with a kinetic reaction assay. The mean CV for alpha-amylase was 4.67%; classrooms were assayed together when possible.

Data transformation.

As with previous studies, cortisol and alpha-amylase at each collection time were markedly skewed; cortisol was strongly positively skewed (kurtosis = 33 to 56) while alpha-amylase was moderately skewed (kurtosis = .13 to 6.27). Other studies (e.g. Fortunato et al., 2008; Gordis et al., 2008) have found similar distributions. At each collection point, cortisol levels were transformed with a natural logarithmic transformation and alpha-amylase levels were subjected to a square root transformation (Tabachnick & Fidell, 2001). The transformations largely normalized the cortisol data (kurtosis = 3.59 to 5.98) and markedly improved the distribution for alpha-amylase (kurtosis = -.39 to .02). After transformations, the data were examined for outliers, defined as greater than or less than three standard deviations from the mean at each of the eight collection points for cortisol and alpha-amylase. Two outliers were identified for alpha-amylase (n = 1 at the second collection; n = 1 at the seventh collection). Concerning cortisol outliers, one child displayed values greater than three standard deviations from the mean at six of the eight collection points; another child displayed values greater than three standard deviations from the mean at two of the eight collection points. Following recommendations from previous studies, these values were replaced with the highest possible value within the specified range (Fortunato et al., 2008; Gordis et al., 2008). Since saliva was collected at similar time points over two days, transformed levels of alpha-amylase and cortisol were averaged at each time point creating an average alpha-amylase and an average cortisol level for each of the four collection times.

To create a summary of individual daily activity of cortisol and alpha-amylase while attending child care, area under the curve with respect to ground (AUC_g) was calculated following the equation recommended by Pruessner, Kirschbaum, Meinlschmid, and Hellhammer (2003). The use of AUC_g accounts for time between saliva collections and allows for total output of activity in the HPA axis and the SNS to be measured without losing numerous degrees of freedom as compared to employing repeated measures. The times of each saliva collection varied by center (due to differences in classroom daily schedules), so a separate equation was computed for each classroom. AUC_g was calculated for children who had data for at least three timepoints following recommendations of previous studies (Saridjan et al., 2010). One child was only present for two collection timepoints and was deleted from further analyses that required AUC_g; this resulted in a sample of 61 children. As in previous studies nontransformed values of cortisol and alpha-amylase were used to calculate AUC_g (Hajat et al., in press). However, AUC_g for cortisol was positively skewed (kurtosis = 51.53), and it was transformed using natural logarithmic transformation (D. Granger, personal communication, May 5, 2010). Further, outliers for AUC_g cortisol were identified ($n = 1$) and the procedure explained above (less than or greater than 3 SD) was utilized. Overall, these transformations markedly improved the distribution for AUC_g cortisol (kurtosis = 3.78). AUC_g for alpha-amylase did not require any transformation as it was normally distributed and outliers were not detected. The transformed AUC_g for cortisol and the nontransformed AUC_g for alpha-amylase were used in all relevant analyses.

CHAPTER V

RESULTS

The primary goal of the study was to examine the patterns of cortisol and alpha-amylase across the day in child care. This study aimed to further contribute to the understanding of children's patterns of cortisol activity (biomarker of activity in the HPA axis) in child care, since previous studies have demonstrated children often show a rise in cortisol throughout the day (Watamura et al., 2009). Concerning alpha-amylase (surrogate biomarker for the SNS), the purpose was largely exploratory as patterns of alpha-amylase for children in child care is unknown. Building on these preliminary aims, the main purpose of this study was to identify the specific indicators of classroom quality that predict individual variation in the overall output of cortisol and alpha-amylase. Additionally, child characteristics such as reactivity and ethnicity have demonstrated links to baseline cortisol and alpha-amylase. Specifically, African-American children may display lower levels of cortisol (Blair et al., 2008) and higher levels of alpha-amylase (Gordis et al., 2008) when compared to Caucasian or Latino children; thus ethnicity was controlled for in all analyses using AUC_g. For ease in interpretation, ethnicity was dichotomized with 47% African American and mixed race and 53% Caucasian and Latino. Further, child negative affect may serve as a moderator between classroom quality and child cortisol activity; there may be a similar moderating relationship between emotion regulation and alpha-amylase. Thus, hierarchical linear models with ethnicity and reactivity at level one and classroom quality at level two were explored predicting individual variation in cortisol or alpha-amylase output.

Descriptives and Preliminary Analyses

Table 2 illustrates the descriptive information for all child level variables. Both transformed and original average cortisol and alpha-amylase at each of the four collection points are shown in Table 2. The mean cortisol levels are within the expected range for healthy, typically developing children (.01μg/dl, microgram per deciliter,to 1.0 μg/dl; McCarthy et al., 2009); standard ranges of alpha-amylase for children are yet to be determined. The child reactivity variables, negative affect and emotion regulation, demonstrated sufficient variability and a normal distribution. Classroom variables also indicated substantial range and variability (Table 3); this is an important precursor for the remaining hypotheses (examining the relationships between cortisol or alpha-amylase and indicators of classroom quality). Regrettably, the Instructional Support domain had a limited range; however this mean is similar to previous large-scale studies (La Paro et al., 2009).

Bivariate correlations were computed for all variables. Five of the classroom quality indicators (Emotional Support, Classroom Organization, Instructional Support, Materials and Activities, Language/Interactions) demonstrated positive relationships with one another (see Table 4). For instance, classrooms with higher Emotional Support were also higher in Language/Interactions. Surprisingly, square footage available per child was negatively related to all of the other classroom variables which was contrary to the expectation that higher quality classrooms would provide more space per individual. Brief post-hoc graphs were executed to visually examine the relationship between square footage per individual and the classroom quality variables. Examination of the five scatterplots revealed a mostly quadratic relationship between the variables. For example, the association between square footage per individual and Language/Interactions is shown in Figure 1 and illustrates that classrooms with median square footage per individual levels demonstrated higher Language/Interactions. With regard to child level associations, children with higher negative affect were likely to attend classrooms of lower Classroom Organization, Instructional Support, Materials and Activities, and Language/Interactions, but more space available per individual. Further, there is a positive association between child emotion regulation and classroom Instructional Support, Materials and Activities, Language/Interactions, but a negative relationship between emotion regulation and square footage per individual. Child AUC_g cortisol was unrelated to any classroom or child variables. However, child AUC_g alpha-amylase was negatively associated with classroom Emotional Support, Materials and Activities, and Language/Interactions indicating higher scores on these indicators of classroom quality were associated with lower child alpha-amylase output throughout the day at child care.

Individual Patterns Across the Day

The first research question explored children’s basic patterns of activity in cortisol and alpha-amylase across the day. All of the following analyses used the transformed variables for cortisol or alpha-amylase. To first examine individual cortisol patterns, average cortisol levels at each of the four collection times were graphed. This graph revealed that, contrary to the hypothesis, cortisol failed to consistently decline across the day for most individuals (Figure 2). Next, average individual levels of alpha-amylase at each of the four collection times were plotted. As with cortisol, alpha-amylase patterns failed to demonstrate the expected pattern of increase across the day (Figure 3). In general, both plots generally revealed inconsistent patterns of activity over the day for individuals. To further explore this relationship, cortisol and alpha-amylase were aggregated at each of the four collection times across individuals, and Figure 4 illustrates the average pattern of activity for all children in this study. It is interesting to note that as cortisol increases at time three, alpha-amylase decreases. This rise in cortisol at time three could be related to the variety of demands situated within daily classroom activities. During this time, 64% of children in this study had just engaged in outdoor play. Overall, this symmetrical relationship between cortisol and alpha-amylase provides preliminary evidence that the HPA axis and SNS are dependent, supporting Bauer et al.'s (2002) additive model of functioning in the stress response system. The remaining analyses utilized AUC_g for cortisol and alpha-amylase to reflect total output for each individual over the course of the day in child care.

Hierarchical Linear Modeling

To explore the four remaining research questions, Hierarchical Linear Modeling (HLM; Raudenbush & Bryk, 2002) was employed in the following analyses given the nested nature of the data (children nested within classrooms). As dictated by the hypotheses, separate models were executed predicting AUC_g cortisol and AUC_g alpha-amylase to explore the main effect of child ethnicity and reactivity, the main effect of classroom characteristics, and any interactions between child reactivity and classroom quality. Due to limited power given the sample size (Spybrook, Raudenbush, Congdon, & Martínez, 2009) each of the six indicators of classroom quality were tested in separate models and results were considered significant if $p < .10$ (Adam & Gunnar, 2001). Negative affect and emotion regulation were centered at the mean in SPSS 17.0 to aid interpretation of results. Lastly, as ethnicity was a control variable, all models were constrained to test only the main effect of ethnicity for each individual.

Cortisol.

To provide support for exploring differences in child cortisol output due to individual (level 1) or classroom (level 2) influences, an unconditional model (AUC_g cortisol = $\gamma_{00} + u_{0j} + r_{ij}$) was computed to examine the amount of variation in child cortisol that lies within and between classrooms where γ_{00} = grand mean of cortisol, u_{0j} = random error, and r_{ij} = child variance. Thirty-two percent of individual variation in cortisol was due to differences between classrooms. Given significant unexplained variation in the unconditional model ($\chi^2 = 40.41$ $p < .001$), a random intercept model was executed to test for the main effect of ethnicity and negative affect (AUC_g cortisol = $\gamma_{00} + \gamma_{10}(\textit{ethnicity}) + \gamma_{20}(\textit{negative affect})\ u_0 + r$). Contrary to the hypothesis neither ethnicity nor negative affect emerged as a significant main effect for individual AUC_g cortisol or contributed to variance within classroom (see Table 5). Following model building recommendations from Raudenbush and Bryk (2002), ethnicity was dropped from future models.

However, one of the aims of this study was to explore the potential interaction between negative affect and classroom quality. Thus even though there was not a main effect of negative affect, subsequent models were built to determine interactions between classroom quality and negative affect. To explore if child negative affect moderated the relationship between classroom quality and child cortisol output, a full model was built to test the main effect of negative affect as well as the moderating effect. Six models were built to explore the relationships for each indicator of quality (e.g. $\mathrm{AUC_g}$ cortisol = γ_{00} + γ_{01}(*emotional support*) + γ_{10}(*negative affect*) + γ_{11}(*emotional support**negative affect*) u_0 - + u_1(*negative affect)* + *r*). Child negative affect was removed from the models as it failed to significantly predict total cortisol as a moderator. Finally, six models were executed exploring the relationship between classroom quality (level two) and child cortisol output (e.g. $\mathrm{AUC_g}$ cortisol = γ_{00} + γ_{01}(*Emotional Support*)). The models failed to find any significant relationships between classroom quality and individual overall cortisol output (see Table 5). Thus, child total cortisol output was not impacted by child negative affect, ethnicity, or classroom quality.

Alpha-amylase.

The unconditional model ($\mathrm{AUC_g}$ alpha-amylase = γ_{00} + u_{0j} + r_{ij}) revealed that 86% of individual variation in alpha-amylase was due to differences in overall alpha-amylase between *classrooms* and significant variance was left to be explained (χ^2 = 21.81, p = .058); thus a random intercept model was built to test the main effects of child emotion regulation and ethnicity ($\mathrm{AUC_g}$ alpha = γ_{00} + γ_{10}(*ethnicity*) + γ_{20}(*emotion regulation*) + u_0 + u_2(*emotion regulation*) + *r*). Results parallel findings from the cortisol models as neither ethnicity nor emotion regulation predicted differences in alpha-amylase output (see Table 6). Following model building recommendations from Raudenbush and Bryk (2002), ethnicity was dropped from the model.

However, to explore (a) the moderating effect of child emotion regulation on the relationship between classroom quality and alpha-amylase output and (b) the main effect of classroom quality on alpha-amylase output, six further models were built (e.g. AUC_g alpha-amylase = $\gamma_{00} + \gamma_{01}$(*emotional support*) + γ_{10}(*emotion regulation*) + γ_{11}(*emotional support* emotion regulation*) $u_0 + u_1$(*emotion regulation)* + *r*). Child emotion regulation was removed from the models as it failed to significantly predict differences in total alpha-amylase and inclusion of emotion regulation in the models markedly decreased model reliability.

Finally, six models predicting AUC_g alpha were built with classroom quality at level two, exploring the main effect of classroom quality on differences in alpha-amylase output. Results revealed that three indicators of classroom quality (Emotional Support, Language/Interactions, and Materials and Activities) significantly predicted individual alpha-amylase output. The final models predicting AUC_g alpha from classroom quality are displayed in Table 6. These results indicate that children in classrooms with higher values for Emotional Support, Language/Interactions, and Materials and Activities displayed lower alpha-amylase output on average (see Figures 5, 6, and 7 respectively for an illustration). Further, the model including classroom Emotional Support explained 63% of the variability of average child alpha-amylase output while the models with Language/Interactions and Materials and Activities contributed to 32% and 41%, respectively. Individual indicators of classroom quality emerged as significant predictors of activity in the SNS.

CHAPTER VI

DISCUSSION

Much of the previous research indicates that children demonstrate a rise in afternoon cortisol while in child care regardless of global quality (Watamura et al., 2009). However, the majority of these research studies have overlooked indicators of quality, creating composites and total scores to measure classroom quality, ignoring the potential relationship between specific indicators of classroom quality (e.g. amount of materials and activities, emotional climate) and cortisol levels. Further, to the author's knowledge, this is the first study to examine alpha-amylase levels of children in child care. The results from this study indicate the importance of measuring activity in the HPA axis and the SNS in order to fully understand functioning in the stress response system in young children.

In contrast to previous research, the current study did not find evidence of a rise in afternoon cortisol for children in child care. Instead, the results from this study provide support for a preliminary body of evidence suggesting that preschool children do not consistently demonstrate a rise in afternoon cortisol at child care (Ouelett-Morin et al., 2009). In this study, both morning and afternoon cortisol levels appeared to decrease. However, there was evidence that cortisol increased at time three, indicating a deviation in the expected diurnal pattern of cortisol (Gunnar & Donzella, 2002). This increase may be explained by an increase in individual physical activity (Hansen, Blangsted, Hansen, Søgaard, & Sjøgaard, 2010) as 64% of children participated in outdoor play before the third collection. Future studies should examine this potential relationship between outdoor play, physical activity, and cortisol levels. Further, examining the differences in individual slopes between time two and time three, physical activity levels, and quality of the outdoor environment may help identify potential relationships to explain the increase in cortisol at time three. However, it is important to note that by the fourth collection, cortisol levels decreased, displaying a more typical pattern of activity in the HPA axis.

Results from this study indicate support for Bauer et al.'s (2002) additive model, as when cortisol increases, it appears that alpha-amylase decreases (see Figure 3). Previous studies with adolescents have found that symmetrical patterns of activity in cortisol and alpha-amylase in response to a stressor predict fewer behavior and emotional problems (Gordis, Granger, Susman, & Trickett, 2006). However, this is the first study to examine cortisol and alpha-amylase in the child care setting, and the findings support measurement of the HPA axis *and* SNS in future studies. Next steps are to identify classroom and individual characteristics that support symmetry in the stress response system.

The primary aim of the current study was to understand how indicators of classroom quality and individual child differences influenced total activity in the HPA axis and SNS. The results from this study provide evidence to partially support Bronfenbrenner's Process-Person-Context-Time model (Bronfenbrenner & Morris, 2006), and thus his theory is used to frame the discussion for the remaining results.

Process

In the classroom, proximal processes take the form of teacher-child interactions and child-environment interactions (Bronfenbrenner & Morris, 2006). Classroom quality defined by Instructional Support (CLASS Pre-K) and Language/Interactions (ECERS-R) examine teacher-child proximal processes. Instructional Support did not emerge as a predictor of overall cortisol or alpha-amylase output in preschool children. It is possible these null findings were a result of the limited range of Instructional Support actually provided in the classrooms; which is also evident in other large scale studies (La Paro et al., 2009). Additionally, the behaviors captured under Instructional Support require children to engage in higher-order thinking and problem solving. Engagement in these types of proximal processes may be a situational stressor for the child, thereby not impacting overall activity in the stress response system. Instead, these types of interactions may cause a temporary increase in cortisol. However, classrooms which also encompass supportive elements such as positive peer relationships, child-led teaching strategies, and a warm sensitive teacher as indicated by higher classroom Emotional Support as well as higher classroom Instructional Support, may best support functioning in the stress response system. Previous research has found similar relationships between teacher's instructional skills versus emotional support and relationships to child outcomes (Lisonbee et al., 2008; Mize & Granger., 2005; Peisner-Fienberg et al., 2001).

Children enrolled in classrooms with increased opportunities to engage in language and conversation displayed significantly lower alpha-amylase output. However, the relationship to overall cortisol output was not significant. With respect to alpha-amylase, there is preliminary evidence suggesting that alpha-amylase may be related to active coping and positive behaviors (Fortunato et al., 2008). Thus, children in classrooms which are rich in language and conversation may be able to engage in more positive behaviors, displaying a typical pattern of alpha-amylase activity. On the other hand, activity in the HPA axis may be more situation-specific and reflect differences in individual experience rather than the average classroom experience (Dougherty, Klein, Congdon, Canli, & Hayden, 2010). Thus, future studies should examine individual child experiences in care, noting stressors or experiences which cause distress to further explore relationships between cortisol and classroom quality. In this study, the measures of classroom quality captured the average classroom experience; not classroom quality at the individual child level.

The opportunity for child-environment proximal processes was captured under Materials and Activities (ECERS-R). While total cortisol output was not related to the amount of materials and planned activities in the classroom, alpha-amylase output was related to these classroom characteristics. Specifically, children in classrooms with more available toys and stimulating activities displayed lower total alpha-amylase. As with Language/Interactions, it could be that increased choice in activities and stimulating opportunities for engagement provide a positive environment, which supports children's coping and emotion management and results in typical alpha-amylase levels. Cortisol, as previously stated, may be elevated in specific classroom situations (e.g. an argument between children over how to build a block tower) but these situations were not captured in the current study.

Proximal processes within the classroom that offer a variety of environmental manipulations and are rich in language and conversation seem to support SNS functioning. Moreover, characteristics of the classroom context may influence the opportunity for proximal processes to occur, thus also influencing activity in the stress response system. Thus, classroom contextual characteristics of Emotional Support, Classroom Organization, and square footage per individual were explored in this study.

Context

The overall emotional climate in the classroom defined by a preponderance of positive relationships, a responsive and supportive teacher, and support for individual autonomy was predictive of lower child alpha-amylase output. These findings support results from Fortunato and colleagues (2008) in that classrooms with more emotional support and positive behaviors may allow children to engage in more positive behaviors. Future studies should explore the specific types of positive behaviors and the strategies teachers use to model emotion regulation to further understand activity in the SNS. However, classroom Emotional Support did not predict individual cortisol output. Emotional Support in this study captured the average child experience in the classroom, and cortisol levels may differ by individual experience and moment-to-moment stressors (Dougherty et al., 2010). Studies that investigate the specific teacher behaviors that support child emotion regulation after experiencing a stressor may better illustrate the relationship between emotional support and child cortisol levels. For example, children's cortisol levels may be lower if, when a stressor is experienced, the teacher is sensitive, acknowledges the child's emotion, and assists in discovering coping mechanisms (Bronson, 2000; Zeidner, Matthews, Roberts, & MacCann, 2003).

To explore Bronfenbrenner's conceptualization of chaos (Bronfenbrenner & Evans, 2000) it was hypothesized that children in classrooms with higher Classroom Organization would display lower alpha-amylase and cortisol output. However, there was not support for this hypothesis. Classroom Organization, as defined by the CLASS Pre-K, is comprised of three separate dimensions: behavior management, productivity, and instructional learning formats. While behavior management and productivity are important to classroom functioning and management, the instructional learning formats dimension captures the ability of the teacher to facilitate learning opportunities using the materials in the classroom. For alpha-amylase output, instructional learning formats may support a child's ability to engage in stimulating and supported opportunities, supporting activity in the SNS. Cortisol levels on the other hand, are often linked to increases in child externalizing behaviors (Gordis et al., 2008). Thus, concerning activity in the HPA axis, it is possible that the strategies the teacher uses to manage and promote positive behavior (e.g. use of proactive strategies, clear behavioral expectations) are important to consider.

It was hypothesized that crowded classrooms, as defined by the amount of square feet available to each individual, would be linked to increased response in the stress response system. However, the amount of square feet available per person did not predict differences in child cortisol or alpha-amylase. In previous studies the amount of useable play space predicted differences in children's cortisol levels (Legendre, 2003) as children with more useable play space had lower cortisol levels. In the current study the total area of the classroom was considered, not the amount of useable play space. Thus, the negative correlation between square feet/individual and the five remaining indicators of classroom quality may be due to the inability of this variable to capture the amount of useable space. Anecdotally, it was noted that larger classrooms had more materials, shelves, and tables, which may have limited the amount of useable play space available for children. This theory supports the quadratic relationship between square footage per individual and the other classroom quality indicators (Figure 1), as classrooms with median square footage may have a balance between square feet/individual and useable place space. Further investigation into this quadratic relationship may aid in identifying the effectiveness of the classroom layout and the best use of space.

Person

The current study examined demand (ethnicity) and force (reactivity) characteristics as predictive of individual differences in cortisol and alpha-amylase output. However, neither person characteristic emerged as a significant predictor of activity in the stress response system. Previous studies have largely examined reactivity in response to a stressor to capture the differences in cortisol (Gunnar, Talge, & Herrera, 2010) and alpha-amylase (Fortunato et al., 2008) levels as the child recovers from the stressor. Since the current study did not use laboratory stressors, but instead captured total activity, it may be that relationship qualities and not individual differences in reactivity are important to understand individual differences in the stress response system. Further, results from Adam and Gunnar (2001) support the hypothesis that total cortisol output across the day is impacted by relationship quality and not individual differences. Future studies should examine the perceived closeness of the teacher-child relationship; these relationship qualities may be salient predictors of total activity in the stress response system across the day in child care (Lisonbee et al., 2008).

This study also failed to find support for Belsky's differential susceptibility hypothesis (1997, 2009). According to differential susceptibility, the quality of the classroom environment may be more important for some children, given individual differences in behavioral reactivity. However, the HLM models exploring these hypotheses were not significant. Previous studies finding support for differential susceptibility have measured reactivity in infancy to predict child outcomes (Crockenberg & Leerkes, 2003; Pluess & Belsky, 2009). Thus, future studies should employ a longitudinal approach, measuring reactivity in infancy and classroom environments across early childhood to understand the relationship between reactivity and the development of the stress response system. Moreover, another theory of conditional adaption, biological sensitivity to context (Boyce & Ellis, 2005), also argues that understanding the environment in infancy is central to predicting children's reactivity. It is possible that early home and child care environments are most important to parse out differences in child cortisol and alpha-amylase activity.

Limitations

There were a few limitations in this study largely due to the small sample size and number of individuals in each classroom. Given the current sample, significant findings were unlikely (Spybrook et al., 2009). It should be noted that even with the small sample size, significant findings emerged providing support that specific indicators of structural and process quality predict differences in children's total alpha-amylase output. However, it is possible that a larger sample size and more children per classroom would be better equipped to discover support for differential susceptibility and biological sensitivity to context, given that moderator variables often require increased power to detect (McClelland & Judd, 1993).

Additionally, there are various measurement techniques designed to understand the activity in the HPA axis and SNS. The current study measured activity using AUC_g to understand total output of cortisol and alpha-amylase. However, other studies have utilized a different manipulation technique: subtracting afternoon cortisol from morning cortisol (Sims et al., 2006); both of these approaches are acceptable and useful to understand activity in the stress response system (Rohleder & Nater, 2009). Further, a longitudinal approach may best yield true relationships between the classroom environment, child characteristics, and activity in the stress response system measured by cortisol and alpha-amylase levels. This approach would also strengthen the bioecological theoretical framework by including the final component of the PPCT model: time (Bronfenbrenner & Morris, 2006). Ideally, a research design which follows infants through the first years of life, capturing the quality of home and child care classrooms, would afford investigation into the BSC and differential susceptibility hypotheses as well as identify classroom, home, and child characteristics that best support functioning in the stress response system.

Conclusion and Implications

This study was the first to examine both cortisol and alpha-amylase in the context of child care. Further, there was considerable variability in child ethnicity, teacher ethnicity, family income, child care quality, and teacher's experience and education, which is absent from much of the current literature (Geoffroy et al., 2006). Child alpha-amylase total output was lower in classrooms with higher Emotional Support, Language/Interactions, and Materials and Activities. Following replication of these findings, professional development opportunities should encourage and support classrooms to maintain high frequencies of these classroom behaviors. Given the current findings, there are two areas that warrant future inquiry in the field of early education and development: teacher-child ratios and peers in the classroom.

In the current study, classrooms with increased conversation, learning opportunities, and activities best supported activity in the SNS. The availability of these interactions for each child may be dependent on the number of adults in the classroom. For instance, classrooms are able to demonstrate more emotional support when more adults are present (NICHD, 2002). Thus, lower teacher-child ratios may afford increased opportunities for teacher-child interactions. Previous research indicates that more positive caregiving occurs in classrooms with lower teacher-child ratios (Shim, Hestenes, & Cassidy, 2004; NICHD, 1996). It is possible that classrooms with lower teacher-child ratios are more likely to have increased opportunities to expose children to emotion language, assist in self-regulation techniques, and provide more individualized support. These types of interactions would support functioning in the stress response system.

Beyond teacher-child ratios, the child care experience for each individual is impacted by the other children in the classroom (Wishard, Shivers, Howes, & Ritchie, 2003). A child must interact with peers to utilize materials in the classroom, and the child may compete with peers for teacher attention. Moreover, children view peers as an important component of their child care experience (Ceglowski & Bacigalupa, 2007), and the influence of peers on classroom quality is largely unknown. Professional development opportunities need to acknowledge the importance of peers as well as highlight techniques by which teachers are able to promote positive peer relationships in the classroom. Preliminary evidence supports the use of teacher-directed scaffolding to support and develop peer relationships in the classroom (Williams, Mastergeorge, & Ontai, 2010).

Focusing on the impact of peers and the importance of low teacher-child ratios in the child care classroom may support optimal child functioning in the stress response system. Additionally, educating teachers and parents about the importance of classroom quality and its relationship to child functioning in the stress response system is imperative. By continuing to parse out the relationships between individual differences, classroom quality, and the functioning of the stress response system, research can identify the mechanisms that support positive child outcomes.

REFERENCES

Abercrombie, H. C., Kalin, N. H., Thurow, M. E., Rosenkranz, M. A., & Davidson, R. J. (2003). Cortisol variation in humans affects memory for emotionally laden and neutral information. *Behavioral Neuroscience, 117*, 505-516.

Adam, E. K., & Gunnar, M. R. (2001). Relationship functioning and home and work demands predict individual differences in diurnal cortisol patterns in women. *Psychoneuroendocrinology, 26,* 189-208.

Ahadi, S. A., Rothbart, M. K., & Ye, R. (1993). Children's temperament in the US and China: similarities and differences. *European Journal of Personality,7*, 359-377.

Bauer, A.M., Quas, J.A., & Boyce, W.T. (2002). Associations between physiological reactivity and children's behavior: Advantages of a multisystem approach. *Journal of Developmental and Behavioral Pediatrics, 23,* 102-113.

Belsky, J. (1997). Variation in Susceptibility to Environmental Influence: An Evolutionary Argument. *Psychological Inquiry: An International Journal for the Advancement of Psychological Theory, 8*, 182-186.

Belsky, J. (2005). Differential susceptibility to rearing influences: An evolutionary hypothesis and some evidence. In B. Ellis & D. Bjorklund (Eds.), *Origins of the social mind: Evolutionary Psychology and Child Development* (pp. 139–163). New York: Guildford.

Belsky, J., Bakermans-Kranenburg, M. J., & van Ijzendoorn, M. H. (2007). For better and for worse: Differential susceptibility to environmental influences. *Current Directions in Psychological Science, 16*, 300-304.

Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B., & Williams, R. (2009). Vulnerability genes or plasticity genes?. *Molecular Psychiatry, 14*, 746-754.

Belsky, J., & Pluess, M. (2009). The nature (and nurture?) of plasticity in early human development. *Perspectives on Psychological Science, 4*, 345-351.

Belsky, J., & Steinberg, L. (1978) The effects of daycare: A critical review. *Child Development, 49,* 929-949.

Blair, C., Granger, D. A., Kivlighan, K. T., Mills-Koonce, R., Willoughby, M., Greenberg, M. T., et al. (2008). Maternal and child contributions to cortisol response to emotional arousal in young children from low-income, rural communities. *Developmental Psychology, 44*, 1095-1109.

Bohus, B., de Kloet, E.R., Veldhuis H. D. (1982). Adrenal steroids and behavioral adaptation: relationship to brain corticoid receptors. In D. Granten, & D. W. Pfaff (Eds.), *Current Topics in Neuroendocrinology* (pp. 107–48). Berlin: Springer-Verlag.

Boyce, W. T., & Ellis, B. J. (2005). Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Development and Psychopathology, 17*, 271-301.

Brake, W. G., Zhang, T. Y., Diorio, J., Meaney, M. J., Gratton, A., 2004. Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *European Journal of Neuroscience, 19,* 1863–1874.

Bronfenbrenner, U. (1979). *The ecology of human development: Experiments by nature and design.* Cambridge, MA: Harvard University Press.

Bronfenbrenner, U., & Morris, P.A. (1998). The ecology of developmental processes. In W. Damon (Series Ed.) & R.M. Lerner (Vol. Ed.), *Handbook of child psychology: Vol. 1. Theoretical models of human development* (5th ed., pp. 993-1028). New York: Wiley.

Bronfenbrenner, U., & Evans, G. W. (2000). Developmental science in the 21st century: Emerging questions, theoretical models, research designs and empirical findings. *Social Development, 9*, 115-125.

Bronfenbrenner, U., & Morris, P. A. (2006). The bioecological model of human development. In W. Damon & R. M. Lerner (Eds.), *Handbook of child psychology* (6th ed., Vol. *Vol. 1. Theoretical models of human development* pp. 793-828). Hoboken, NJ: John Wiley & Sons.

Bronson, M. B. (2000). *Self-regulation in early childhood: Nature and nurture*. New York: Guilford Press.

Burchinal, M., Howes, C., Pianta, R., Bryant, D., Early, D., Clifford, R., et al. (2008). Predicting child outcomes at the end of kindergarten from the quality of pre-kindergarten teacher-child interactions and instruction. *Applied Developmental Science, 12*, 140-153.

Burchinal, M. R., Roberts, J. E., Riggins, R., Jr., Zeisel, S. A., Neebe, E., & Bryant, D.

(2000). Relating quality of center-based child care to early cognitive and language development longitudinally. *Child Development*, *71,* 339-357.

Calkins, S. D., & Fox, N. A. (2002). Self-regulatory processes in early personality development: A multilevel approach to the study of childhood social withdrawal and aggression. *Development and Psychopathology, 14,* 477-498.

Calkins, S. D., & Howse, R. B. (2004). Individual differences in self-regulation: Implications for childhood adjustment. In P. Philippot & R. S. Feldman (Eds.), *The regulation of emotion* (pp. 307-332). Manwah, New Jersey: Lawerence Erlbaum Associates.

Capizzano, J., & Adams, G. (2000). *The number of child care arrangements used by children under five: Variations across states.* Urban Institute Washington, DC.

Cassidy, D. J., Hestenes, L. L., Hansen, J. K., Hegde, A., Shim, J., & Hestenes, S. (2005). Revisiting the two Faces of child care quality: Structure and process. *Early Education & Development, 16*, 505-520.

Ceglowski, D. A., & Bacigalupa, C. (2007). "[I] play a lot": Children's perceptions of child care. *Journal of Research in Childhood Education, 22*, 173-188.

Charmandari, E., Tsigos, C., & Chrousos, G. (2005). Endocrinology of the stress response. *Annual Review of Physiology, 67,* 259–284.

Chatterton, R. T., Jr., Vogelsong, K.M., Lu, Y.C., Ellman, A., B., & Hudgens, G.A. (1996). Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clinical Physiology, 16,* 433-448.

Clinton, S., Miller, S., Watson, S. J., & Akil, H. (2008). Prenatal stress does not alter innate novelty-seeking behavioral traits, but differentially affects individual differences in neuroendocrine stress responsivity. *Psychoneuroendocrinology, 33,* 162-177.

Chryssanthopoulou, C. C., Turner-Cobb, J. M., Lucas, A., & Jessop, D. (2005). Childcare as a stabilizing influence on HPA axis functioning: A reevaluation of maternal occupational patterns and familial relations. *Developmental Psychobiology, 47*, 354-368.

Crockenberg, S. C., & Leerkes, E. M. (2005). Infant temperament moderates associations between childcare type and quantity and externalizing and internalizing behaviors at years. *Infant Behavior and Development*, *28*, 20-35.

Cronbach, L. J. (1951). Coefficient alpha and the internal structure of tests. *Psychometrika, 16,* 297-334.

Cryer, D. (1999). Defining and assessing early childhood program quality. *Annals of the American Academy of Political and Social Science, 563,* 39-55.

Cryer, D., Tietze, W., & Wessels, H. (2002). Parents' perceptions of their children's child care: a cross-national comparison. *Early Childhood Research Quarterly, 17*, 259-277.

Davis, E. P., & Granger, D. A. (2009). Developmental differences in infant salivary alpha-amylase and cortisol responses to stress. *Psychoneuroendocrinology, 34,* 795-804.

Dettling, A. C., Gunnar, M. R., & Donzella, B. (1999). Cortisol levels of young children in full-day childcare centers: Relations with age and temperament. *Psychoneuroendocrinology, 24*, 519-536.

Dettling, A.C., Parker, S.W., Lane, S., Sebanc, A., & Gunnar, M.R. (2000). Quality of care and temperament determine changes in cortisol concentrations over the day for young children in childcare. *Psychoneuroendocrinology, 25*, 819-836.

Dougherty, L. R., Klein, D. N., Congdon, E., Canli, E., & Hayden, E. P. (2010). Interaction between 5-HTTLPR and BDNF Val66Met polymorphisms on HPA axis reactivity in preschoolers. *Biological Psychology, 83*, 93-100.

Ellis, B. J., Essex, M. J., & Boyce, W. T. (2005). Biological sensitivity to context: II. Empirical explorations of an evolutionary-developmental theory. *Development and Psychopathology, 17,* 303-328.

El-Sheikeh, M., Erath, S. A., Buckhalt, J. A., Granger, D. A., & Mize, J. (2008). Cortisol and children's adjustment: The moderating role of sympathetic nervous system activity. *Journal of Abnormal Child Psychology, 36,* 601-611.

El-Sheikh, M., Mize, J. & Granger, D.A. (2005). *Endocrine and parasympathetic responses to stress predict child adjustment, physical health, and cognitive functioning.* Paper presented at the biennial meeting of the Society for Research in Child Development, Atlanta, GA. April.

Evans, G. W. (2006). Child development and the physical environment. *Annual Review of Psychology, 57,* 423-451.

Evans, G.W., & Saegert, S. (2000). Residential crowding in the context of inner city poverty. In S. Wapner, J. Demick, T. Yamamoto, & H. Minami (Eds.), *Theoretical perspectives in environment-behavior research: Underlying assumptions, research problems, and methodologies.* (pp. 247-268). New York: Kluwer Academic/Plenum Publishers.

Fortunato, C. K., Dribin, A. E., Granger, D. A., & Buss, K. A. (2008). Salivary alpha-amylase and cortisol in toddlers: Differential relations to affective behavior. *Developmental Psychobiology, 50,* 807-818.

Geoffroy, M. C., Cote, S. M., Parent, S., & Seguin, J.R. (2006). Daycare attendance, stress, and mental health. *Canadian Journal of Psychiatry, 51,* 607-615.

Gordis, E. B., Granger, D. A., Susman, E. J., & Trickett, P. K. (2008). Salivary alpha amylase-cortisol asymmetry in maltreated youth. *Hormones and Behavior, 53*, 96-103.

Gordis, E. B., Granger, D. A., Susman, E. J., & Trickett, P. K. (2006). Asymmetry between salivary cortisol and alpha-amylase reactivity to stress: Relation to aggressive behavior in adolescents. *Psychoneuroendocrinology, 31*, 976-987.

Granger, D.A., & Fortunato, C.K. (2008). Integration of salivary biomarkers and analytes into developmental science: A critical review of theory, tactics, and strategies. Manuscript submitted for publication.

Granger, D. A., Kivlighan, K. T., Blair, C., El-Sheikh, M., Mize, J., Lisonbee, J. A., et al. (2006). Integrating the measurement of salivary alpha-amylase into studies of child health, development, and social relationships. *Journal of Social and Personal Relationships, 23*, 267-290.

Granger, D. A., Schwartz, E. B., Booth, A., Curran, M., Zakaria, D. (1999). Assessing dehydroepiandrosterone in saliva: A simple radioimmunoassay for use in studies of children, adolescents and adults. *Psychoneuroendocrinology, 24,* 567-579.

Granger, D. A., Stansbury, K., & Henker, B. (1994). Preschoolers' behavioral and neuroendocrine responses to social challenge. *Merrill-Palmer Quarterly, 40,* 190-211.

Greenspan, S. I. (2003). Child care research: A clinical perspective. *Child Development, 74*, 1064-1068.

Gunnar, M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology, 27*, 199-220.

Gunnar, M. R., Kryzer, E. M., Phillips, D., & Vandell, D. (2001). *Modified observational ratings of the caregiving environment (M-ORCE).* Unpublished coding instrument and manual.

Gunnar, M. R., Talge, N. M., & Herrera, A. (2010). Stressor paradigms in developmental studies: What does and does not work to produce mean increases in salivary cortisol. *Psychoneurendocrinology, 34,* 953-967.

Gunnar, M. R., Tout, K., de Hann, M., Pierce, S., & Stansbury, K. (1997). Temperament, social competence, and adrenocortical activity in preschoolers. *Developmental Psychobiology, 31*, 65-85.

Gunnar, M., & Quevedo, K. (2007). The Neurobiology of Stress and Development. *Annual Review of Psychology, 58,* 145-173.

Gustafsson, P. E., Janlert, U., Theorell, T., & Hammarström, A. (2010). Life-course socioeconomic trajectories and diurnal cortisol regulation in adulthood. *Psychoneuroendocrinology, 35,* 613-623.

Hajat, A., Diez-Roux, A., Franklin, T. G., Seeman, T., Shrager, S., Ranjit, N., et al. (2010). Socioeconomic and race/ethnic differences in daily salivary cortisol profiles: The Multi-Ethnic Study of Atherosclerosis. *Psychoneuroendocrinology*. Advance online publication. doi: 10.1016/j.psyneuen.2009.12.009

Hansen, Å. M., Blangsted, A. K., Hansen, E. A., Søgaard, K., & Sjøgaard, G. (2010). Physical activity, job demand–control, perceived stress–energy, and salivary cortisol in white-collar workers. *International Archives of Occupational and Environmental Health, 83,* 143-153.

Harmon, A. G., Hibel, L. C., Rumyantseva, O, & Granger, D. A. (2007). Measuring salivary cortisol in studies of child development: Watch out—what goes in may not come out of saliva collection devices. *Developmental Psychobiology, 49,* 495-500.

Harms, T., Clifford, R.M., Cryer, D. (1998). *Early Childhood Environmental Rating Scale-Revised*. New York: Teachers College Press.

Hill, J. L., Waldfogel, J., Brooks-Gunn, J., & Han, W. (2005). Maternal employment and child development: A fresh look using newer methods. *Developmental Psychology, 41,* 833-850.

Hill-Soderlund, A. L., Mills-Koonce, W. R., Propper, C., Calkins, S. D., Granger, D. A., Moore, G. A., et al. (2008). Parasympathetic and sympathetic responses to the strange situation in infants and mothers from avoidant and securely attached dyads. *Developmental Psychobiology*, *50*, 361-376.

Johnson, J.O. (2002). *Who's Minding the Kids? Child Care Arrangements: Winter 2002.* Current Population Reports, P70-101. U.S. Census Bureau, Washington, DC, 2005.

Keane, S.P., & Calkins, S.D. (2004). Developmental trajectories of early behavior problems: Implications for kindergarten social status. *Journal of Abnormal Child Psychology*, *32*, 409-423.

Kirschbaum, C., & Hellhammer, D. H. (1989). Salivary cortisol in psychobiological research: An overview. *Neuropsychobiology, 22,* 150–169.

Kyrou, I., & Tsigos, C. (2009). Stress hormones: physiological stress and regulation of metabolism. *Current Opinion in Pharmacology, 9,* 787-793.

La Paro, K. M., Hamre, B. K., Locasale-Crouch, J., Pianta, R. C., Bryant, D., Early, D., et al. (2009). Quality in kindergarten classrooms: Observational evidence for the need to increase children's learning opportunities in early education classrooms. *Early Education and Development, 20,* 657-692.

La Paro, K. M., Pianta, R. C., & Stuhlman, M. (2004). The Classroom Assessment Scoring System: Findings from the pre-kindergarten year. *The Elementary School Journal, 104,* 409-426.

Layzer, J. I., & Goodson, B. D. (2006). The "Quality" of Early Care and Education Settings: Definitional and Measurement Issues. *Evaluation Review, 30*, 556-576.

Legendre, A. (2003). Environmental features influencing toddlers' bioemotional reactions in day care centers. *Environment and Behavior, 35,* 523-548.

Levine, S. (1994). The ontogeny of the hypothalamic–pituitary–adrenal axis: The influence of maternal factors. *Annals of the New York Academy of Sciences, 746,* 275–288.

Lisonbee, J. A, Mize, J., Payne, A. L., & Granger, D. A. (2008). Children's cortisol and the quality of the teacher-child relationships in child care. *Child Development, 79,* 1818-1832.

Loman, M. M., & Gunnar, M. R. (2010). Early experience and the development of stress reactivity and regulation in children. *Neuroscience and Biobehavioral Reviews, 34,* 867-876.

Martimportugues-Goyenechea, C., & Gomez-Jacinto, L. (2005). Simultaneous multiple stressors in the environment: Physiological stress reactions, performance, and stress evaluation. *Psychological Reports, 97,* 867-874.

McCarthy, A. M., Hanrahan, K., Kleiber, C., Zimmerman, M. B., Lutgendorf, S., & Tsalikian, E. (2009). Normative salivary cortisol values and responsivity in children. *Applied Nursing Research, 22,* 54-62.

McClelland, G.H., & Judd, C.M. (1993). Statistical difficulties of detecting interactions and moderator effects. *Psychological Bulletin, 114,* 376-390.

McEwen, B. S., & Seeman, T. (1999). Protective and damaging effects of mediators of stress: Elaborating and testing the concepts of allostasis and allostatic load. *Annals of the New York Academy of Sciences, 896,* 30-47.

Meaney, M. J., Diorio, J., Francis, D.,Widdowson, J., La Plante, P., Caldui, C., Sharma, S., Seckl, J., Plotsky, P. (1996). Early environmental regulation of forebrain glucocorticoids receptor gene expression: Implications for adrenocortical responses to stress. *Developmental Neuroscience, 18,* 49-60

Mize, J., Lisonbee, J., & Granger., D. A. (2005). *Stress in child care: Cortisol and alpha-amylase may reflect different components of the stress response.* Presented at the Biennial Meeting of Society for Research in Child Development. Atlanta, GA.

Nater, U. M., Rohleder, N., Schlotz, W., Ehlert, U., & Kirschbaum, C. (2007). Determinants of the diurnal course of salivary alpha-amylase. Psychoneuroendocrinology, 32(4), 392-401.

NICHD Early Child Care Research Network (1996). Characteristics of infant child care: Factors contributing to positive caregiving. *Early Childhood Research Quarterly, 11*, 269–306.

NICHD ECCRN (2001). Child care and children's peer interaction at 24 and 36 months: The NICHD study of early child care. *Child Development, 72,* 1478-1500.

NICHD ECCRN (2002). The relation of global first-grade classroom environment to structural classroom features and teacher and student behaviors. *The Elementary School Journal, 102,* 367-387.

NICHD ECCRN (2003). Does Quality of Child Care Affect Child Outcomes at Age 4 1/2? *Developmental Psychology*, *39*, 451-469.

Nicolson, N. A. (2004). Childhood parental loss and cortisol levels in adult men. *Psychoneuroendocrinology, 29*, 1012-1018.

Nigg, J. T. (2006). Temperament and developmental psychopathology. *Journal of Child Psychology and Psychiatry, 47*, 395–422

Ouellet-Morin, I., Boivin, M., Dionne, G., Lupien, S. J., Arsenault, L., Barr, R. G., et al. (2008). Variations in heritability of cortisol reactivity to stress as a function of early familial adversity among 19-month-old twins. *Archives of General Psychiatry, 65*, 211-218.

Ouellet-Morin, I., Tremblay, R. E., Boivin, M., Meaney, M., Kramer, M., & Côté, S. M. (2010). Diurnal cortisol secretion at home and in child care: A prospective study of 2-year-old toddlers. *Journal of Child Psychology and Psychiatry*, *51,* 295-303.

Palacios R., Sugawara I. (1982). Hydrocortisone abrogates proliferation of T cells in autologous mixed lymphocyte reaction by rendering the interleukin-2 Producer T cells unresponsive to interleukin-1 and unable to synthesize the T-cell growth factor. *Scandinavian Journal of Immunology, 15,* 25–31.

Palkovits M. (1987). Organization of the stress response at the anatomical level. In E. de Kloet,V. Wiegant, & D. de Wied, (Eds.), *Progress in Brain Research* (pp. 47–55). Amsterdam: Elsevier Science.

Phillipsen, L. C., Burchinal, M. R., Howes, C., & Cryer, D. (1997). The prediction of process quality from structural features of child care. *Early Childhood Research Quarterly, 12*, 281-303.

Pianta, R.C., LaParo, K.M., & Hamre, B.K. (2007). *Classroom Assessment Scoring System: Pre-K.* Baltimore, MD: Brooks Publishing.

Rappolt-Schlichtmann, G., Willett, J. B., Ayoub, C. C., Lindsley, R., Hulette, A. C., & Fischer, K. W. (2009). Poverty, relationship conflict, and the regulation of cortisol in small and large group contexts at child care. *Mind, Brain, and Education, 3*, 131-142.

Raudenbush, S.W., & Bryk, A.S. (2002). *Hierarchical linear models: Applications and data analysis methods* (2nd ed.). Thousand Oaks, CA: Sage Publications, Inc.

Rohleder, N., & Nater, U. M. (2009). Determinants of salivary alpha-amylase in humans and methodological considerations. *Psychoneuroendocrinology, 34*, 469-485.

Romeo, R., & McEwen, B. S. (2006). Stress and the adolescent brain. *Annals of the New York Academy of Sciences, 1094,* 202–214.

Rothbart, M.K., Ahadi, S.A., Hershey, K.L., & Fisher, P. (2001). Investigations of temperament at 3-7 years: The Children's Behavior Questionnaire. *Child Development, 72,* 1394-1408.

Peisner-Feinberg, E. S., Burchinal, M. R., Clifford, R. M., Culkin, M. L., Howes, C., Kagan, S. L., et al. (2001). The Relation of Preschool Child-Care Quality to Children's Cognitive and Social Developmental Trajectories through Second Grade. *Child Development, 72*, 1534-1554.

Porges, S. W., Doussard-Roosevelt, J. A., & Maiti, A. K. (1994). Vagal tone and the physiological regulation of emotion. *Monographs of the Society for Research in Child Development, 59,* 167-186.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 28,* 916-931.

Saridjan, N. S., Huizink, A. C., Koetsier, J. A., Jaddoe, V. W., Mackenbach, J. P., Hofman, A., et al. (2010). Do social disadvantage and early family adversity affect the diurnal cortisol rhythm in infants? The Generation R Study. *Hormones and Behavior*, *57,* 247-254.

Shields, A., & Cicchetti, D. (1997). Emotion regulation among school-age children: The development and validation of a new criterion Q-sort scale. *Developmental Psychology, 33,* 906-916.

Shields, A., & Cicchetti, D. (1998). Reactive aggression among maltreated children: The contributions of attention and emotion dysregulation. *Journal of Child Clinical Psychology, 27,* 381-395.

Shim, J., Hestenes, L, & Cassidy, D. (2004). Teacher structure and child care quality in preschool classrooms. *Journal of Research in Childhood Education, 19,* 143-157.

Spangler, G. (1991). The emergence of adrenocortical circadian function in newborns and infants and its relationship to sleep, feeding and maternal adrenocortical activity. *Early Human Development, 25,* 197-208.

Spinrad, T. L., Eisenberg, N., Granger, D. A., Eggum, N. D., Sallquist, J., Haugen, R. G., et al. (2009). Individual differences in preschoolers' salivary cortisol and alpha-amylase reactivity: Relations to temperament and maladjustment. *Hormones and Behavior*, *56,* 133-139.

Spybrook, J., Raudenbush, S. W., Congdon, R., & Martínez, A. (2009). Optimal design for longitudinal and multilevel research: Documentation for the “Optimal Design” software.

Tabachnick B.G., & Fidell, L.S. (2001). *Using multivariate statistics (4th ed.).* New York: Allyn and Bacon.

Tout, K., de Haan, M., Campbell, E. K., & Gunnar, M. R. (1998). Social behavior correlates of cortisol activity in child care: Gender differences and time-of-day effects. *Child Development, 69,* 1247-1262.

Tudge, J. R. H, Mokrova, I., Hatfield, B. E., Karnik, R. B. (2009). Uses and misuses of Bronfenbrenner’s bioecological theory of human development. *Journal of Family Theory and Review, 1,* 198-210.

Vermeer, H. J., & van Ijzendoorn, M. H. (2006). Children's elevated cortisol levels at daycare: A review and meta-analysis. *Early Childhood Research Quarterly, 21,* 390-401.

Volkmann, E.R., & Weekes, N.Y. (2006). Basal SIgA and cortisol levels predict stress-related health outcomes. *Stress & Health, 22,* 11-23.

Wachs, T. D. (1993). Nature of relations between the physical and social microenvironment of the two-year-old child. *Early Development and Parenting, 2,* 81-87.

Watamura, S. E., Donzella, B., Alwin, J., & Gunnar, M. R. (2003). Morning-to-Afternoon Increases in Cortisol Concentrations for Infants and Toddlers at Child Care: Age Differences and Behavioral Correlates. *Child Development, 74,* 1006-1020.

Watamura, S.E., Kryzer, E. M., & Robertson, S.S. (2009). Cortisol patterns at home and child care: Afternoon differences and evening recovery in children attending very high quality full-day center-based child care. *Journal of Applied Developmental Psychology, 30*, 475-485.

Watamura, S. E., Sebanc, A. M., & Gunnar, M. R. (2002). Rising cortisol at childcare: Relations with nap, rest, and temperament. *Developmental Psychobiology, 40,* 33-42.

Williams, S. T., Mastergeorge, A. M., & Ontai, L. L. (2010). Caregiver involvement in infant peer interactions: Scaffolding in a social context. *Early Childhood Research Quarterly*, *25,* 251-266.

Wishard, A. G., Shivers, E. M., Howes, C., & Ritchie, S. (2003). Child care program and teacher practices: Associations with quality and children's experiences. *Early Childhood Research Quarterly, 18,* 65-103.

Zeidner, M., Matthews, G., Roberts, R. D., & MacCann, C. (2003). Development of emotional intelligence: Towards a multi-level investment model. *Human Development, 46,* 69-96.

APPENDIX

TABLES AND FIGURES

Figure 1. Relationship between square foot per individual and Language/Interactions

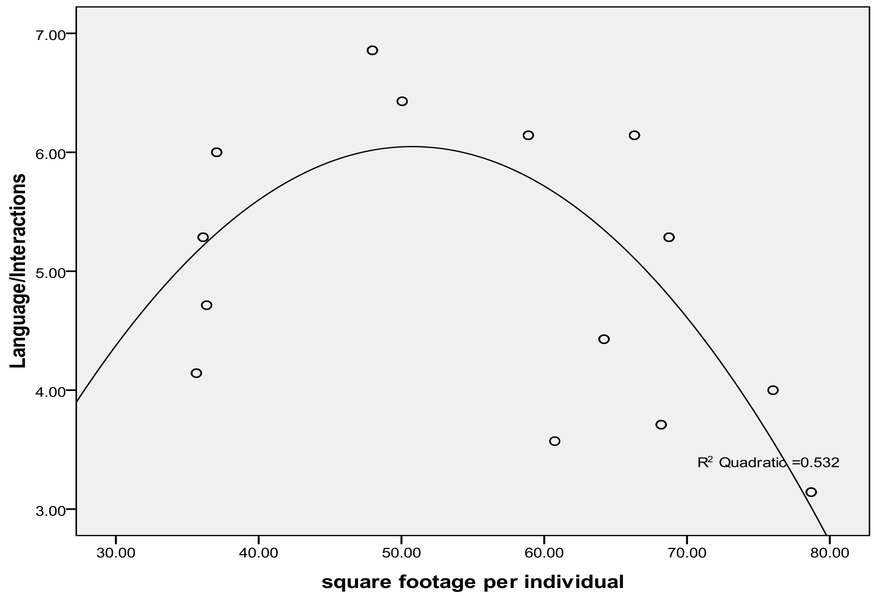

Figure 2. Individual levels of cortisol at each collection

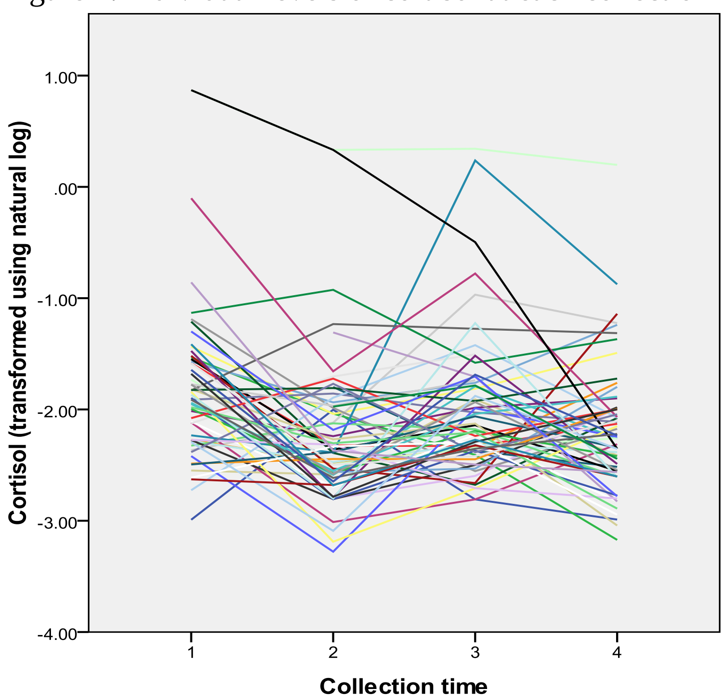

Figure 3. Individual levels of alpha-amylase at each collection

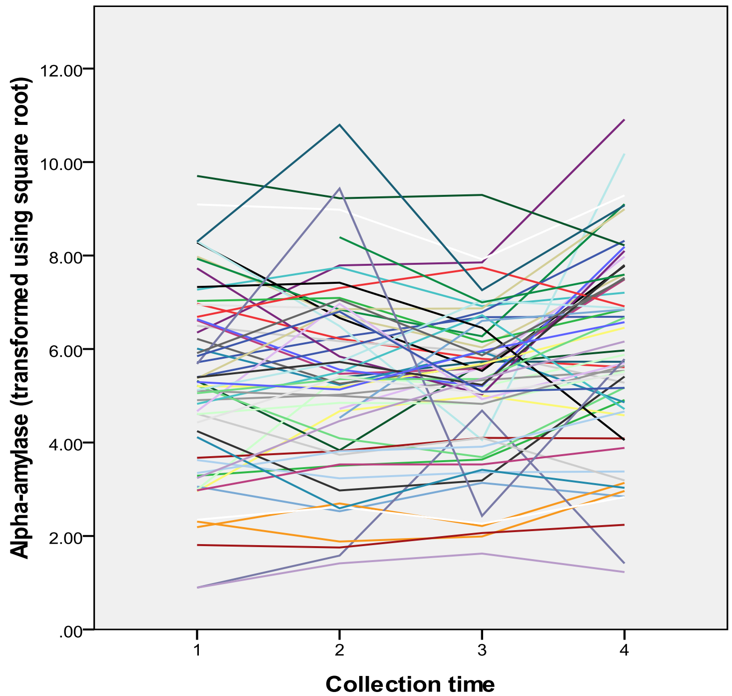

Figure 4. Average levels of alpha-amylase and cortisol at each collection

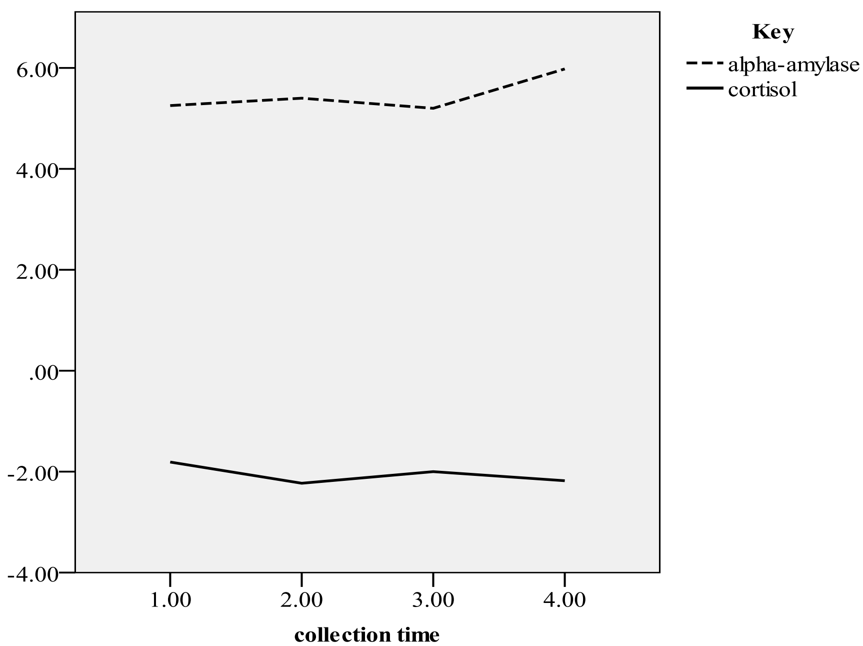

Figure 5. Average child alpha-amylase at each collection by classroom Emotional Support

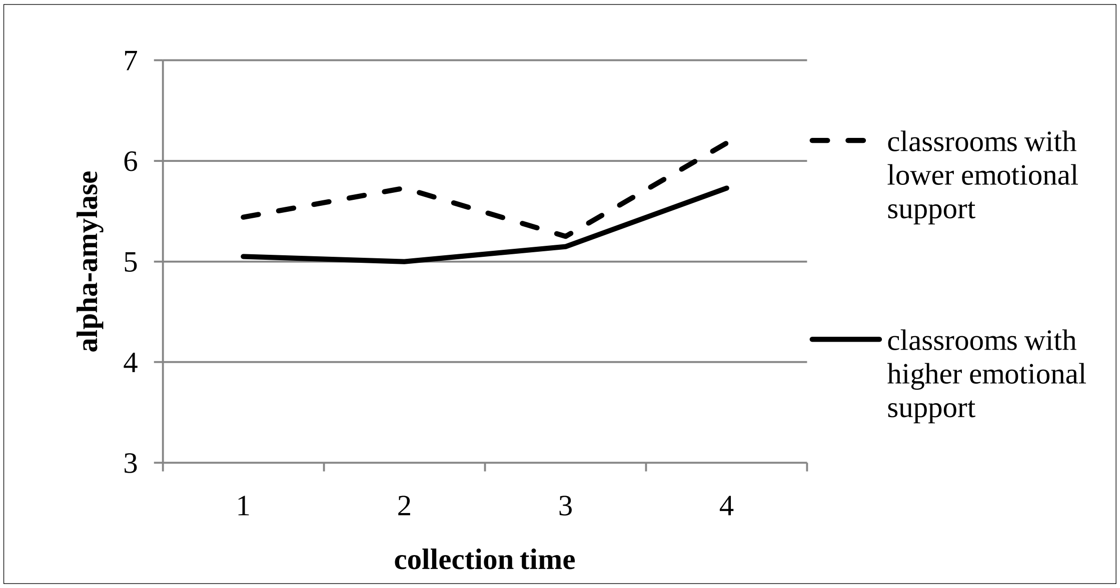

Figure 6. Average child alpha-amylase at each collection by Classroom Activities and Materials

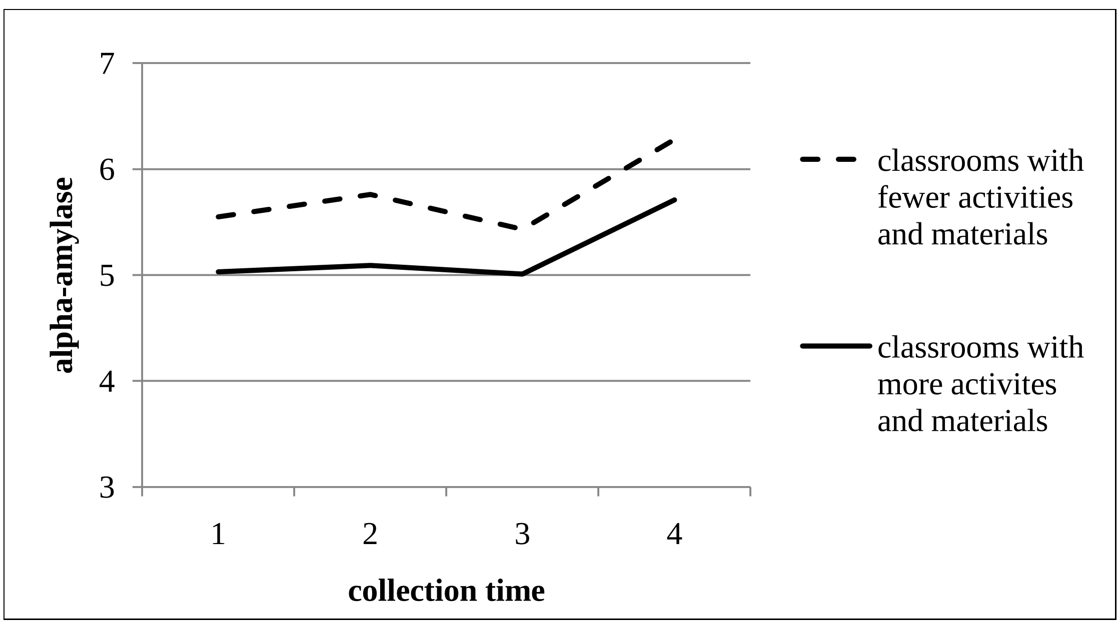

Figure 7. Average child alpha-amylase by classroom Language/Interactions

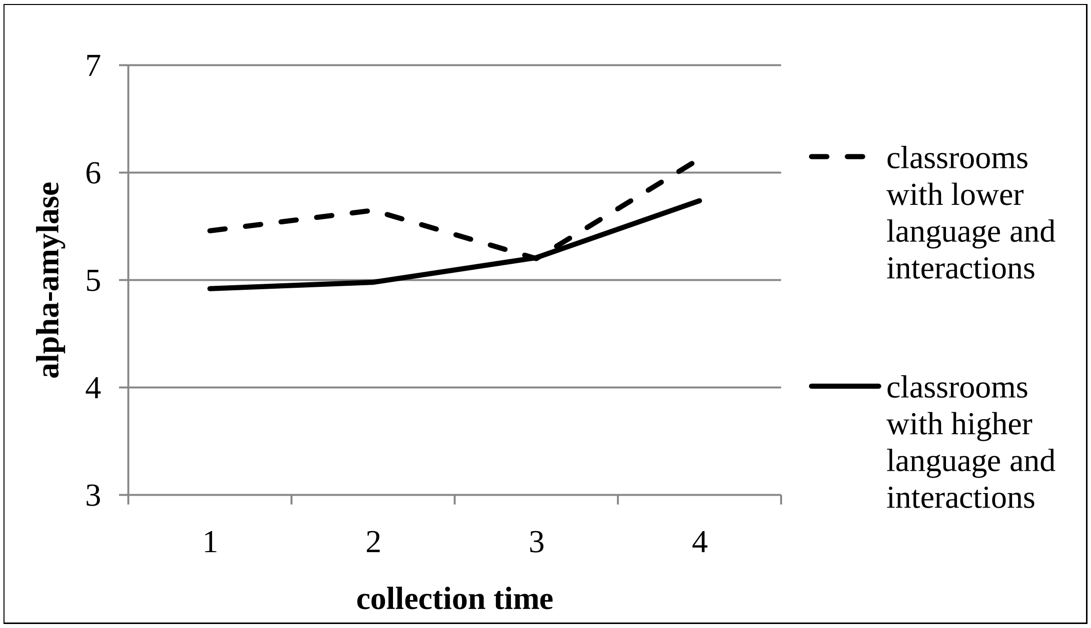

Table 1. Demographics by STAR rating

	High Quality (STAR rating of 4 or 5)	Low Quality (STAR rating of 2 or 3)
Child Characteristics		
Income		
< 24,000	26%	21%
24,000 to 48,000	33%	42%
48,000 to 84,000	15%	16%
> 84, 000	26%	21%
Marital Status		
Married	66%	55%
Separated	12%	15%
Divorced	5%	0%
Single	17%	30%
Child Ethnicity		
African American	37.5%	30%
Caucasian	42.5%	45%
Latino	2.5%	5%
Other/Mixed Race	17.5%	20%
Child Care Subsidy	15%	15%
	***M* (*SD*)**	***M* (*SD*)**
Negative Affect	3.87 (.53)	3.95 (.67)
Emotion Regulation	3.12 (.44)	2.95 (.54)
Hours/week in child care	39.90 (6.8)	41.05 (4.75)
AUC_g Cortisol	4.15 (.66)	3.98 (.25)
AUC_g Alpha-amylase	14,697.23 (8,676.34)	15,379.82 (10,562.96)
Classroom Characteristics		
Emotional Support	5.46 (.76)	4.68 (.61)
Classroom Organization	5.23 (.66)	4.71 (.55)
Instructional Support	2.34 (.65)	1.75 (.49)
Materials and Activities	4.96 (.85)	3.86 (.66)
Language/Interactions	5.48 (.88)	4.18 (1.2)
Square Feet/Individual	54.71 (14.49)	59.93 (15.98)

Table 2. Descriptives for Child Variables

	N	*M (SD)*	*Kurtosis*	*Minimum*	*Maximum*
Cortisol					
Time 1	56	.34 (.89)	33.15	.06	6.03
Time 2	61	.23 (.70)	51.27	.04	5.40
Time 3	61	.24 (.52)	47.21	.06	3.99
Time 4	59	.22 (.73)	57.57	.04	5.73
Time 1[a]	56	-1.81 (.72)	5.63	-2.99	.87
Time 2[a]	56	-2.23 (.66)	6.00	-3.28	.33
Time 3[a]	61	-1.99 (.64)	4.00	-2.81	.34
Time 3[a]	61	-2.18 (.59)	3.59	-3.17	.20
AUC_g[a]	61	4.09 (.56)	3.78	3.25	6.08
Alpha-amylase					
Time 1	57	32.31 (21.81)	.13	.80	94.14
Time 2	61	33.65 (23.53)	2.05	2.00	116.60
Time 3	61	30.92 (18.74)	6.27	2.65	117.26
Time 4	60	40.66 (26.09)	.39	1.66	119.06
Time 1[a]	57	5.25 (2.03)	-.39	.89	9.70
Time 2[a]	61	5.40 (2.02)	.02	1.41	10.80
Time 3[a]	61	5.20 (1.64)	-.05	1.62	9.30
Time 4[a]	60	5.98 (2.14)	-.31	1.23	10.91
AUC_g	61	14,921.03 (9,253.26)	.50	951.08	43,081.73
Negative affect	61	3.90 (.58)	-.15	2.63	5.35
Emotion regulation	61	3.06 (.48)	1.44	1.38	3.88

[a] Transformed values

Table 3. Descriptive Statistics for Classroom Variables

	N	*M (SD)*	*Kurtosis*	*Minimum*	*Maximum*
Emotional Support	14	5.20 (.80)	-.69	3.55	6.58
Classroom Organization	14	5.06 (.68)	.42	3.33	6.28
Instructional Support	14	2.15 (.66)	-1.24	1.20	3.17
Materials & Activities	14	4.60 (.95)	-1.11	3.00	6.11
Language/Interactions	14	5.06 (1.16)	-1.30	3.14	6.86
Square ft/individual	14	56.42 (15.07)	-1.40	35.64	78.70

Table 4. Correlations Between Child and Classroom Variables

	1.	2.	3.	4.	5.	6.	7.	8.	9.
Classroom variables									
1. Emotional Support	--								
2. Classroom Organization	.78**	--							
3. Instructional Support	.80**	.87**	--						
4. Materials and Activities	.64**	.52**	.58**	--					
5. Language/Interactions	.72**	.62**	.75**	.32*	--				
6. Square ft/individual	-.46**	-41**	-.45**	-.46**	-.45**	--			
Child variables									
7. Negative affect	-.20	-.29*	-.35**	-.32*	-.38**	.40**	--		
8. Emotion regulation	.17	.25	.37**	.21	.33**	-34**	-.46**	--	
9. AUC_g cortisol	.08	-.10	-.04	.07	.14	-.21	-.05	.08	--
10. AUC_g alpha-amylase	-.30*	-.14	-.17	-.27*	-.25*	.10	-.08	.18	.04

* $p < .05$
** $p < .01$

Table 5. HLM models predicting AUC_g cortisol

AUC_g cortisol = $\gamma_{00} + \gamma_{10}$*(ethnicity)* + γ_{20}*(negative affect)* $u_0 + r$

Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	4.06	.14	28.08	.00
Ethnicity, γ_{10}	.04	.14	.27	.79
Negative Affect, γ_{20}	.04	.13	.33	.74

AUC_g cortisol = $\gamma_{00} + \gamma_{01}$*(Emotional Support)* + r

Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	4.07	.11	37.67	.00
Emotional Support, γ_{01}	.07	.13	.51	.67

AUC_g cortisol = $\gamma_{00} + \gamma_{01}$*(Classroom Organization)* + r

Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	4.08	.11	37.73	.00
Classroom Organization, γ_{01}	-.05	.16	-.34	.74

AUC_g cortisol = $\gamma_{00} + \gamma_{01}$*(Instructional Support)* + r

Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	4.06	.11	37.41	.00
Instructional Support, γ_{01}	-.02	.17	-.12	.94

AUC_g cortisol = $\gamma_{00} + \gamma_{01}$*(Materials and Activities)* + r

Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	4.08	.11	37.50	.00
Materials and Activities, γ_{01}	.03	.12	.25	.81

AUC_g cortisol = $\gamma_{00} + \gamma_{01}$*(Language/Interactions)* + r

Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	4.07	.11	38.13	.00
Language/Interactions, γ_{01}	.07	.09	.73	.48

AUC_g cortisol = $\gamma_{00} + \gamma_{01}$*(square footage/individual)* + r

Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	4.07	.11	38.13	.00
Square footage/individual, γ_{01}	-.01	.01	-.97	.35

Table 6. HLM models predicting AUC_g alpha-amylase

AUC_g alpha-amylase = $\gamma_{00} + \gamma_{10}$*(ethnicity)* + γ_{20}*(emotion regulation)* $u_0 + r$				
Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	16272.06	1948.16	8.35	.00
Ethnicity, γ_{10}	-2271.55	2534.13	-.90	.37
Emotion regulation, γ_{20}	-1243.99	2689.95	-.46	.65
AUC_g alpha-amylase = $\gamma_{00} + \gamma_{01}$*(Emotional Support)* + r				
Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	15213.28	1255.91	12.11	.00
Emotional Support, γ_{01}	-3404.19	1571.91	-2.17	< .05*
AUC_g alpha-amylase = $\gamma_{00} + \gamma_{01}$*(Classroom Organization)* + r				
Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	15084.67	1453.66	10.38	.00
Classroom Organization, γ_{01}	-1856.31	2154.84	-.86	.41
AUC_g alpha-amylase = $\gamma_{00} + \gamma_{01}$*(Instructional Support)* + r				
Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	15040.73	1316.25	10.45	.00
Instructional Support, γ_{01}	-2280.83	2214.55	-1.03	.32
AUC_g alpha-amylase = $\gamma_{00} + \gamma_{01}$*(Materials and Activities)* + r				
Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	14927.25	1319.18	11.32	.00
Materials and Activities, γ_{01}	-2961.20	1413.86	-1.90	$<.10^t$
AUC_g alpha-amylase = $\gamma_{00} + \gamma_{01}$*(Language/Interactions)* + r				
Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	15082.05	13.49	11.18	.00
Language/Interactions, γ_{01}	-2041.47	1169.67	-1.75	$<.10^t$
AUC_g alpha-amylase = $\gamma_{00} + \gamma_{01}$*(Square footage/individual)* + r				
Fixed Effects	*Coefficient*	*se*	*t Ratio*	*p value*
Intercept, γ_{00}	14962.71	1505.20	9.94	.00
Square footage/individual, γ_{01}	64.81	100.43	0.65	.53

* $p < .05$
^{t} $p < .10$